ERRATUM

In Article 7013, entitled Skin Changes after Subcutaneous Injection of Epinephrine, reference No. 8 should read: Luckhardt, A. B., and Koppanyi, T., *Am. J. Physiol.*, 1927, **81**, 436.

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7032 C*

Possible Prophylactic Value of Bacteriophage Lysed Staphylococcus Cultures in Recurrent Skin Boils in Horses.

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From the Department of Bacteriology and Experimental Pathology, Stanford University.

Recurrent skin boils in saddle horses may present something of a problem. In March, 1932, the Department of Military Science and Tactics of Stanford University requested assistance in checking the spread of cutaneous boils among about 70 saddle horses. Twenty-one horses had already become badly infected. The use of autogenous staphylococcus vaccines and the usual sanitary precautions had failed to keep the disease in check, three-fourths of the horses having become affected. Aerobic and anaerobic cultures of sterilely procured material from the lesions of several of the horses indicated that *Staphylococcus aureus* might be the responsible agent. The Preisz-Nocard bacillus was not isolated. The organisms cultured from the lesions proving bacteriophage susceptible, a quantity of lysate was prepared for prophylactic injections. The 21 horses already infected were given 3 injections of 10 cc. each of the undiluted lysate by the subcutaneous route within a period of 12 days. The remaining 50 horses were given 2 such injections. The lesions in the horses already infected regressed very rapidly so that by the tenth day following the first injection all of the horses were apparently entirely free of boils. A few weeks later one of the inoculated horses developed 2 small boils, but with this exception all of the horses have remained free of boils for more than a year.

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* P represents a preliminary, C a complete manuscript.

In view of the fact that staphylococcus vaccines and other prophylactic measures failed, it would appear that the injections of staphylococcus lysate were directly responsible for the improvement which followed. The condition among these horses subsided too rapidly to be satisfactorily accounted for on other grounds. It appears quite unlikely, however, that such a rôle as the lysate may have played in checking the disease is attributable to a direct lytic or sensitizing action of bacteriophage on the organisms in the lesions, since the lysate was not injected directly into the individual lesions, and probably could not have reached the more distant lesions in a concentration sufficiently high to effect a lytic or sensitizing action on the organisms present in the lesions. It is possible, however, that the results may be attributable to a superior antigenic quality of bacteriophage lysed, as against intact but killed staphylococci used as a vaccine, such lysed cultures containing in addition to bacteriophage, a certain residue of dispersed staphylococcus proteins, as well as certain toxic products of microbic growth, against both of which the body may have to react in establishing an effective immunity. Larkum[1] has recently reported experimental observations which indicate that staphylococcus bacteriophage antigenically behaves like a toxoid, stimulating the formation in animals of an antiserum endowed with antitoxic action for staphylococcus toxins. It seems quite unlikely that the results observed by us are attributable in any important degree to so-called non-specific protein action, since the condition among the horses not only cleared up quickly but permanently.

The observations on these horses, together with the results which have been obtained with lysates in furunculosis in man,[2] satisfy us that whatever the precise mode of action of these lysates may be when injected into the animal body, bacteriophage lysed staphylococcus cultures possess definite virtues in the treatment and prevention of certain pyogenic infections of the skin.

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[1] Larkum, N. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1395.

[2] Schultz, E. W., *California and Western Medicine*, 1933, **37**, No. 1.

7033 P

Cholesterol Esters of the Blood in Experimental Pancreatic Diabetes.*

I. L. CHAIKOFF AND A. KAPLAN.

From the Division of Physiology, University of California Medical School, Berkeley, California.

Bloor *et al*[1] found that the esterified cholesterol in plasma of diabetic patients was slightly higher than normal. It seemed of interest, therefore, to record some preliminary observations on the postabsorptive blood cholesterol of completely depancreatized dogs that have been maintained in this laboratory for varying periods of time by means of insulin and a suitable diet.

The oxidative procedures of Okey[2] have been used for cholesterol determinations. The treatment of these animals has been reported elsewhere.[3] Blood cholesterol determinations on 3 normal dogs are also recorded. Dog 4, normal, is a litter mate of dog 1, diabetic;

TABLE I.
Postabsorptive Whole Blood Cholesterol

Dog	Cholesterol Total mg. per 100 cc.	Free mg. per 100 cc.	Ester mg. per 100 cc.	Ester as % total
1 Airdle Female diabetic	102	95	7	7
2 Wht-c-Brn-Hd Female diabetic	101	102	0	0
3 Brownie Female diabetic	100	97	3	3
4 Airdle Female normal	163	123	40	25
5 Blk Female normal	183	126	57	31
6 1-N-1 Male normal	137	104	33	24

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* The expense of this investigation was defrayed in part by a grant from the Research Board of the University of California, Berkeley. The insulin was generously donated by the Eli Lilly Company.

[1] Bloor, W. R., Buckner, E., Gibbs, C. B. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 63.

[2] Okey, R., *J. Biol. Chem.*, 1930, **88**, 367.

[3] Chaikoff, I. L., and Lachman, G. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 237.

both of these animals have been maintained on the same diet since September 1, 1931, when dog 1 was depancreatized. Dog 5, normal, and dog 2, diabetic, are also litter mates, both of these animals having received the same diets since July 27, 1932, the day on which the latter was depancreatized. The third dog, 1-N-1, was kept on the diet for approximately one month before blood samples were taken.

Typical results are shown in Table I. There is a marked reduction of the ester cholesterol of the fasting blood of depancreatized dogs. Esterified cholesterol was completely absent from the blood of diabetic dog 2. Further investigations to determine the time of onset of these lipid changes following pancreatectomy are in progress.

7034

Accumulation of Ions. Further Proof of Non-Equilibrium Condition in Valonia.*

S. C. BROOKS.

From the Department of Zoology, University of California.

In a previous paper[1] I reported that when living cells of *Valonia macrophysa* were immersed for periods of 2-3 days in sea water to which had been added isotonic NaCl solution (10-50% by volume) there occurred an increase in the concentration of potassium and a decrease in that of sodium relative to that of chloride. Since these changes were in the direction opposite to those made in the composition of the external medium, it was concluded that this experiment furnished *crucial* proof that the normal condition in *Valonia* was not an equilibrium state. The validity of the data has been doubted by Cole on statistical grounds,[2] and by Jacques and Osterhout on experimental grounds.[3] I have therefore repeated my experiments in the endeavor to meet these criticisms.

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* This work was assisted by grants from the National Research Council and from the Board of Research of the University of California, which are gratefully acknowledged. To the Stazione Zoologica at Naples, where I occupied the Woods Hole-Columbia University Table, and to Mr. I. Walker, of Papeete, Tahiti, who helped greatly by giving me facilities and working quarters, I wish also to express my deep indebtedness.

[1] Brooks, S. C., *Protoplasma*, 1929, **8**, 389.

[2] Cole, K. C., *Collecting Net*, 1930, **5**, 32.

Experiments were done on *Valonia macrophysa* at Naples, which tended to confirm my earlier results, but they were unsatisfactory statistically. Recently similar experiments were done on specimens of *Valonia ventricosa* at Tahiti. These specimens were extraordinary in the perfection of their selective permeability to ions: normal sap was found to consist of a KCl solution about 0.61 M and over 99% pure; Ca, Mg, and Na were present in concentrations of about 2, 1, and 1 mM/1, respectively.

Analyses were made by the methods previously described[4] except that K was determined by the cobaltinitrite method of Hibbard and Stout,[5] which was found by appropriate tests to be as accurate as the chloroplatinate method and much quicker. Na, being determined as the difference between total K + Na sulfates and K, is so small as to lie almost within experimental error. The negative values for Na might be due to this alone, or they might be due to a systematic error of less than 1% in one of the 2 directly determined values. The samples are not adequate for direct Na determination.†

TABLE I.

The ratios of K/Cl and Na/Cl in terms of molar concentration as found in the sap of *Valonia ventricosa* after immersion in K-poor sea water for various periods.

Solution contains		K/Cl after immersion for			Na/Cl after immersion for		
Sea water	Isotonic NaCl	3 days	6 days	11 days	3 days	6 days	11 days
10 parts	None	0.981	0.940		0.0017	0.0033	
9 "	1 part	1.005	0.940	0.942	—0.0083	0.0017	0.0017
3 "	1 "	0.949	0.940	0.938	0.0017	0.0083	0.0017
1 "	1 "	0.972	0.905	0.898	—0.0050	0.0050	0.0033

Table I shows that when a relatively slight change was made in the sea water, as in the 9:1 mixture whose K concentration was 0.010 M, and cells were exposed to this solution for only 3 days, the expected increase in the ratio of K/Cl occurred. The decrease in Na/Cl is worth noting, even though the uncertainty as to the figures for Na would make it, if taken alone, unreliable. In experiments lasting 3 days where a greater change was made in the sea water ($K<0.010$ M), which thus correspond to the shortest experiment of Jacques and Osterhout, the result is like theirs in showing decrease in K/Cl. Experiments of duration shorter than 3 days had to be omitted because of lack of material, while those of longer duration again show a decrease in K/Cl. Since Jacques and Osterhout made all their determinations on cells exposed for 3 or more days to a solution containing 0.006 GM/l of K it will be seen that in those of our experiments which were substantially similar we both find a decrease in K/Cl. Unfortunately, they have not reported experiments comparable to those of mine in which I have found the ratio of K/Cl to have increased.‡

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[3] Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1932, **15**, 537.

[4] Brooks, S. C., *Science*, 1933, **77**, 221.

[5] Hibbard, P. L., and Stout, P. R., *J. Assn. Official Agr. Chem.*, 1933, **16**, 137.

† The analyses were performed by Mr. B. Weidenbaum.

The data show an increase in the ratio of K/Cl when the reduction in the K content of sea water is slight and the experiment short, and a fall in that ratio with increase in either factor. The changes shown in Table I are not large, but they are consistent with all my previous findings, and suggest why other experimenters failed to observe the fact that decrease in the K content of sea water does under the right conditions lead to an increase in the proportion of K in the sap.

7035 P

Effects of Dinitrophenol on an Experimental Sarcoma of the White Rat.*

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From the Departments of Obstetrics and Gynecology and of Pharmacology, Stanford University School of Medicine.

Recent studies of the effect of dinitrophenol on metabolism[1] have prompted us to study its effect upon tumor growth. Since it is known that the metabolism of the cell of malignant neoplasms is more active than that of the normal cell, we were curious to learn whether or not dinitrophenol administered either by injection or mixed with the food would demonstrably affect the activity of the neoplastic cell. The tumor material utilized in this study is a fibrosarcoma which kills white rats in from 30 to 60 days. It was de-

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‡ Jacques and Osterhout find practically no change in K/Cl at the time of their first observation (3 days), but the smoothing of the curves in their figures obscures this fact.

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

[1] Tainter, M. L., and Cutting, W. C., *J. Pharm. Exp. Therap.*, 1933, **48**, 410. (See for citation of literature.)

rived from a transplantable fibroma by intensive inbreeding. The fibroma, in turn, is a derivative from the mammary adenofibroma of the white rat. The sarcoma is non-metastatic but recurs locally for indefinite periods. It otherwise behaves like a malignant tumor of connective tissue origin.

Thirty white rats varying in age from 84 to 110 days were divided into 6 equal groups and implanted with a sarcoma from the same donor. Two groups were left untreated for controls. Two groups were injected twice daily, with 15 mg. per kg. body weight of alpha-dinitrophenol, the dose being increased when necessary to produce a maximum temperature rise of 2°C. One of these 2 groups was injected from the time of implantation and the other 14 days after the implant was made, or 2 days after active growth was apparent. One group was injected with 1% sodium bicarbonate, the solvent used in making the solution of dinitrophenol, and one group was fed stock diet to which had been added dinitrophenol in the concentration of 1:1000 (daily dose of dinitrophenol ingested about 43 mg. per kg.). The period of observation was between 3 and 4 weeks. The following results were obtained:

Gross Changes. As was to be expected, the average gain of body weight of all dinitrophenol-treated animals was retarded. The tumors of animals fed dinitrophenol grew with about the same speed as those of the controls. Animals injected with dinitrophenol from the time of implantation showed a slight retardation of growth of the tumor when compared to tumors in animals injected after the implant had begun to grow actively. On gross examination, neither group showed any remarkable changes not observed in the controls or animals injected with sodium bicarbonate.

Microscopic Changes. Cytologic appearances of tumors from animals injected with or fed dinitrophenol were not uniform. They differed from the controls and animals injected with sodium bicarbonate only in so far as most of them showed a marked vascular stasis, an apparent reduction in numbers of mitotic figures, an increase in active cellular destruction beneath the zone of growth, as expressed by pyknosis and karyolysis, and the presence of an increased number of leukocytes. The cellular destruction is not necessarily the direct result of the administration of dinitrophenol, but most likely depends upon the speeding up of surface growth which might be regarded as part of the general increase in cellular metabolism of the host as a whole. This contention of an increased speed of surface growth is strengthened by the presence of areas of increased vascularity in this zone.

The avidity of the cells of the growth zone may therefore be suspected of being increased when dinitrophenol is administered.

Conclusions. Dinitrophenol did not affect macroscopically the growth of an experimental sarcoma in rats, but microscopically there were evidences of increased vascularity and destructive cellular changes, whose significance remains undetermined.

7036 P

Effect of Agitation Upon Bacterial Growth.

C. S. MUDGE AND F. R. SMITH.

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The course of population growth in a bacterial culture is known to depend upon many environmental factors, such as temperature, food supply, reactions, etc. We wish to suggest another factor which, from our studies, seems to be of importance—shaking.

The apparatus used in the shaking consists of a motor-driven shaft to which clamps can be attached. The motion of the shaft is reciprocal. The clamps are used for securing culture flasks to the machine. We have generally used about 275 shakes per minute. The extreme agitation is attained in flasks having an up and down excursion of 2.5 inches. For the minimum shaking, the center of mass of the medium was placed at the center of motion of the shaft, thus obtaining little more than a rippling of the surface of the medium.

Flasks inoculated with similar amounts of *E. coli* culture have been studied. Plates were prepared at intervals; smears at the time plates were made. Control flasks unshaken were also studied.

The results indicated in the accompanying figure: (a) The agitated culture reaches a higher population level compared to the unagitated. (b) The growth rate is virtually the same (generation time agitated 26 minutes, unagitated 30 minutes). (c) Log phase lengthened (in some instances 100% longer). (d) Cell size greatest at beginning of log phase, least at end. (e) Cell size 1/3 less in agitated culture.

The observation was also made that (a) Agitated cells (less in size) much more motile than the non-agitated cells. This appeared to be a Brownian movement. (b) Degree of agitation not a factor in phenomenon.

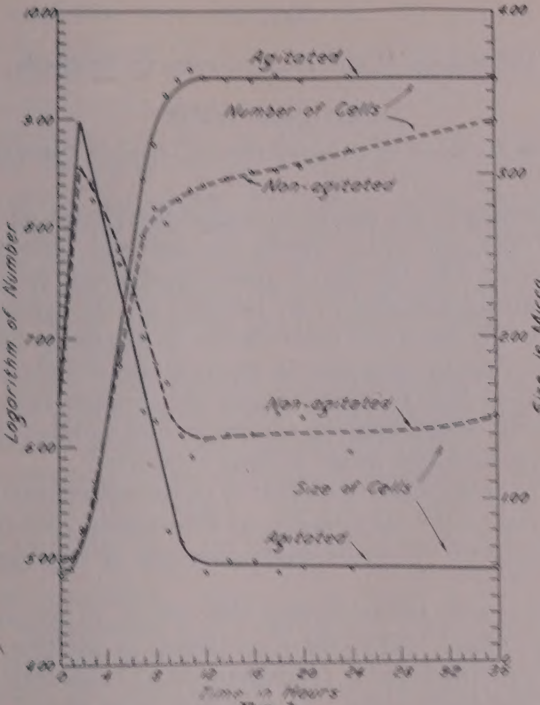

FIG. 1.

Showing the effect of agitation upon the population growth of a culture of *E. coli*, together with the size of the organisms in the agitated and unagitated cultures.

Our results are similar to those reported by Winslow, Walker and Sutermeister,[1] for their studies on aeration. It might seem in the light of their work that our results reflect the aeration due to the agitation.

Doubtless aeration does play a part in these observations. That it is not the whole story must be admitted when it is known that we have observed this phenomenon (but to a lesser degree it is true) when flasks were agitated under a vacuum as well as under oil. We have not as yet studied the effect of replacement of air by nitrogen. We have no explanation for what we have observed. Whether it is due to the fact that the agitated cells are prevented from adsorbing end products or not we do not know. It would appear to be a possibility.

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[1] Winslow, C.-E. A., Walker, H. H., and Sutermeister, Margaret, *J. Bacteriol.*, 1933, **24**, 185.

7037 P

Dinitrophenol and Regeneration in Tubularia.

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From the School of Biological Sciences, Stanford University.

Recent studies of the effects of alphadinitrophenol on the higher vertebrates and man[1] have shown its powerful influence on the metabolic rate. The fact that it resembles thyroxin in this respect has led to an examination of its influence on the regenerative development of certain lower invertebrates of relatively simple structure, some of whose responses to thyroxin are already known. It will be recalled that thyroxin retards the division rate of animal cells, the early development of sea urchin and ascidian[2] and regeneration of the hydranth in the hydroid *Pennaria.*[3]

During the past summer the effect of alphadinitrophenol in various concentrations on the regeneration of the hydroid *Tubularia crocea* has been observed. A single experiment will be considered at this time.

Four dishes were prepared, each with 100 cc. of sea water. One served as a control. The others contained, in addition to the sea water, alphadinitrophenol in concentrations of 1:100,000, 1:200,000 and 1:400,000 respectively. In each dish were placed 30-32 segments, 15-20 mm. long, from the distal ends of an equal number of

TABLE I.

Dishes	I		II		III		IV	
Hour 0	32 segments in 1:100,000 DNP		32 segments in 1:200,000 DNP		30 segments in 1:400,000 DNP		30 segments in sea water	
18	To sea water; no hydranths		To sea water; no hydranths		To sea water; no hydranths		To sea water; no hydranths	
	Regen.	Het	Regen.	Het	Regen.	Het	Regen.	Het
40	12	3	13	0	25	0	26	0
46	20	3	25	0	28	0	29	0
48	26	6	27	4	28	1	29	0
64	32	16	32	19	30	7	30	4
73	32	21	32	21	30	7	30	5

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[1] Tainter, M. L., and Cutting, W. C., *J. Pharm. Exp. Ther.*, 1933, **49**, 410; Cutting, W. C., Mehrtens, H. G., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 193.

[2] Torrey, H. B., Riddle, M. C., and Brodie, J. L., *J. Gen. Physiol.*, 1925, **7**, 449; Torrey, H. B., *Carneg. Inst. Year Book*, 1927, **26**, 228; *Endocrinology*, 1928, **12**, 65.

[3] Torrey, H. B., *Carneg. Inst. Year Book*, 1928, **27**, 286.

Tubularia stems that were similar in size and general condition. Eighteen hours later, and before any hydranths had appeared, they were all transferred to sea water. Subsequent events that bear especially on the present report are recorded in Table I.

This table shows not only an initial *lag* in the appearance of hydranths on the pieces exposed to the higher concentrations of alphadinitrophenol but an equally marked *acceleration* in the appearance of hydranths heteromorphically. During the next 24 hours, all stems had produced hydranths at one end at least. Those in dishes I and II had overhauled the others in this respect while retaining their earlier advantage in number of heteromorphic hydranths, even increasing it.

To what shall we refer these curiously contrasting results?

When heteromorphosis occurs in *Tubularia,* the distal hydranth appears, typically, in advance of the proximal; also, in the presence of a well developed distal hydranth the regeneration of the proximal hydranth is materially retarded. There is an initial distal-proximal polarity that is much less pronounced when the primary hydranth is removed, and may be nullified or even reversed by one of several experimental procedures. When, for instance, the distal ends are exposed to deoxygenated water, the proximal ends to well aerated water, the proximal hydranths now develop not only more rapidly than the distal, but more rapidly than would have been the case had not the development of the distal hydranth been inhibited by lack of oxygen.[4]

It seems possible to regard the immediate effect of alphadinitrophenol as depressing to development at both ends of each piece sufficiently not only to slow down during exposure the development of the distal hydranth but by so doing to lessen the influence of the latter on proximal regeneration. This would explain the fact that on the return of the pieces to sea water development proceeded on somewhat more even terms.

Like thyroxin, then, alphadinitrophenol depresses developmental processes in such lower organisms as *Tubularia,* processes which are relatively independent of the exceptional oxidation which it has been shown to promote in mammals.

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[4] Torrey, H. B., *Univ. Calif. Publ. Zool.*, 1912, 9, 249.

7038 P

Temperature Regulation in Baby Chicks.

M. KLEIBER AND C. WINCHESTER.

From the College of Agriculture, University of California, Davis.

At environmental temperatures below 25° C. baby chicks of one to 3 weeks of age huddle together when they are not eating. This procedure may decrease their loss of heat and is then to be regarded as a type of temperature regulation. As it would be a matter of a higher biological unit than the single animal, it could be classified as social temperature regulation. It would be superimposed on the individual regulation, within which Rubner distinguished the chemical regulation at low and the physical regulation at high environmental temperatures. The social temperature regulation would be physical in its nature but effective only at low environmental temperatures, that is, in the realm of the chemical regulation.

In order to determine whether such a possible influence really exists, the fasting metabolism (24 to 36 hours after the last feed) of baby chicks was determined in groups of 10 individuals, which were allowed to come together in one trial and were separated by divisions of chicken wire in the next trial. With another group, the test was first made with the chicks separated, and was followed by the trial during which they were allowed to huddle together. At an environmental temperature of 14°-15° C., both groups showed a reduction of the metabolism of 15% as a result of huddling.

In similar experiments conducted at a high environmental temperature (37.8° C.) however, the baby chicks have an even higher fasting metabolism when they are allowed to come together than when they are separated. This is shown in Table I, where the heat

TABLE I.
Influence of Huddling on Metabolism of Baby Chicks

Air Temperature °C	No. of Chicks per group	Age of Chicks days		Body weight W. 10^3 (gm.)		Heat Production Calories per $W^{3/4}$		Together in % of Separated
		Separated	Together	Separated	Together	Separated	Together	
37.8	10	19	17	84.2	79.9	90	94	104
”	10	18	20	47.6	47.5	77	86	112
21	5	21	23	107.3	110.1	144	131	91
”	5	22	20	101.4	96.1	149	141	95
”	5	18	16	87.9	89.2	148	146	98
15.5	10	22	20	116.3	117.0	179	151	85
13.9	10	19	21	105.6	102.4	194	166	86

production determined in 12 hours during the night is calculated per 24 hours and per unit of the three-fourths power of the average body weight.

These last experiments are in agreement with the studies of Benedict and coworkers on the influence of huddling on the metabolism as a possible source of error in respiration trials with groups of animals. They conclude that huddling together does not affect the heat production of mice or rats[1] or sheep.[2] These animals seem to have been tested above their critical temperatures, where they produce more heat than they need for maintaining their body temperatures, and where, consequently, a reduction of the metabolism by social temperature regulation cannot be expected.

Our experiments indicate that huddling together may be a means of saving energy at low environmental temperatures.

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[1] Benedict, F. G., and Fox, E. L., *Pflüger's Arch.*, 1933, **231**, 470.

[2] Benedict, F. G., and Ritzman, E. G., *Arch. für Tierernährung und Tierzucht.*, 1931, **5**, 60.

Minnesota Section.

University of Minnesota, October 18, 1933.

7039 P

Serum Lipid Changes and Therapeutic Effects of Various Oils in Infantile Eczema.

ARILD E. HANSEN. (Introduced by Irvine McQuarrie.)

From the Department of Pediatrics, University of Minnesota.

In a previous communication[1] the iodine number of the serum fatty acids was reported to be abnormally low in infantile eczema during the active phase of the disease. Additional data have confirmed the original findings. The average iodine number of the serum fatty acid in 21 determinations on 11 cases of infantile eczema was 82, while that of 21 determinations on 11 normal infants in the same age group was 114. The magnitude of the difference between these 2 groups is similar to that between rats suffering from unsaturated fatty acids deficiency and normal rats.[2]

In December, 1931, oils especially rich in unsaturated fatty acids were given to 2 infants suffering with severe eczema, with good clinical results. Twelve additional cases treated in like manner since that time have shown a similar response. This paper reports changes in the iodine number of the serum fatty acids in 4 cases which could be kept under close observation over a period of months while receiving treatment. All 4 received the same diet and same local care of the skin. Three received supplements of linseed or corn oil while the fourth received none.

The iodine number of the serum fatty acids was subnormal in all 4 cases before treatment was instituted, but increased to normal levels, in cases RS, DL and BS coincidentally with the marked clinical improvement. (Fig. 1.) In contrast to this response was that of the infant RB. This control subject, receiving no oil supplement, failed to show definite clinical improvement. The iodine number not only did not rise during the long period of observation but

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[1] Hansen, Arild E., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1198.

[2] Hansen, Arild E., and Burr, Geo. O., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1201.

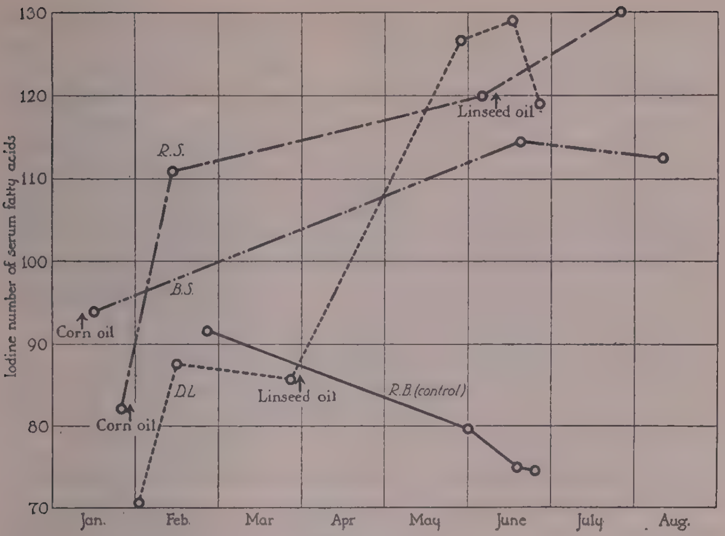

FIG. 1.
Effect of oil supplements on the iodine number of serum fatty acids in infantile eczema.

actually fell slightly. Infant DL was carefully observed in the hospital for about 3 months for any sign of a spontaneous remission before being given oil. No improvement occurred during this period and the iodine number remained low. Three weeks after oil was added to the diet, however, the skin became clear and the iodine number rose to the normal level as it had done in the other 2 treated cases.

7040 P

Possible Mechanism of Crude Coal Tar Therapy in Infantile Eczema.*

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From the Department of Pediatrics, University of Minnesota.

The therapeutic value of ointments containing crude coal tar in infantile eczema has long been recognized. It is not infrequently noted in eczema cases showing extensive skin eruption that untreated areas, as well as those to which direct application of the tar is made, improve. This suggests that crude coal tar may have a systemic as well as a local effect.

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* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

In the light of the observations that the serum fatty acids of infants with eczema are less unsaturated than those of normal subjects of the same age[1] and that administration of oils high in unsaturated fatty acids causes an increase in the degree of unsaturation as well as a clinical improvement in the condition,[2] a preliminary study of the effect of local applications of tar ointment on the fatty acids of the serum has been made. Data from 3 cases followed over a period of several months are presented here.

Case 1, an eczematous male infant 15 months of age, showed values of 334 and 322 mg. iodine absorbed per 100 cc. of serum on 2 successive days at the height of an outbreak of eczema. Eight days after local treatment with crude coal tar ointment the patient had improved clinically and the iodine absorption had risen to 486. One and 2 months later the values were 494 and 520 respectively and the condition remained good. In subsequent cases the iodine number of the serum fatty acids was studied.

Case 2, an infant 5 months of age with severe eczema, was found during the active phase of the disease to have an iodine number of the serum fatty acids ranging between 79 and 81. After application of coal tar ointment for one week, which caused marked improvement in the condition of the skin, the iodine number was found to be 109. At the end of the second week of treatment it was 95 and two weeks later was 101. Active treatment was then omitted for 3 weeks, during which the skin remained entirely clear, except for the appearance of a slight eruption in the folds of the neck. The iodine number at the end of this period was 113.

Case 3, a 6-months-old infant with severe eczema showed an iodine number of the serum fatty acids of 78 before the ointment was applied. This had risen to 96 at the end of a 10-day period of treatment during which the lesions showed marked improvement. Four weeks after the tar was stopped the iodine number had again fallen to 84 and in 5 weeks it was found to be 76. Coincidently with the fall in iodine number, there was a recurrence of the skin lesions. Tar was reapplied and in one week the iodine number of the serum fatty acids had risen to 93 and one week later to 103. At the end of the third week it was still 101. At the end of the fifth week the treatment was discontinued but there was no recurrence of the eczema. One month after discontinuance of treatment the iodine number was found to be 97 and 2 weeks later still it was 112, the skin remaining clear. The patient was discharged from the hospital in good condition. Ten days later she returned with a marked generalized eruption and the iodine number determined at the height of eruption was 83.

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[1] Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1198.

[2] Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 160.

The tentative conclusion drawn from these preliminary studies is that the mechanism by which external application of crude coal tar effects healing in infantile eczema has much in common with that observed when oils containing unsaturated fatty acids are fed. In both instances clinical improvement is accompanied or preceded by a return of the iodine number of the serum fatty acids from subnormal to normal levels.

7041 P

Adrenal Insufficiency and Capacity for Sustained Work Output.

D. J. INGLE. (Introduced by F. H. Scott.)

From the Psychological Laboratories, University of Minnesota.

During the past 2 years the writer has been investigating work output of adrenalectomized rats with the object of evaluating work capacity as a criterion of adrenal cortex insufficiency. Other questions which we hope to answer are: is the medulla necessary to maintain capacity for normal work? will the injection of extracts completely replace the rôle of the normally functioning cortex? and, is nervous innervation necessary for the normal secretory activity of the cortex? Several findings seem to be reliably established and to deserve report.

We have previously made a brief report on methods.[1] The gastrocnemius muscle of an intact animal is loaded with 100 gm. and is forced to contract 3 times per second by faradic stimulation. The animal is anesthetized with sodium luminal. Our setup allows temperature and humidity control. Four animals may be worked simultaneously, the electrodes to each animal being connected in series allowing the same impulse to stimulate each animal. Automatic recording devices are used. Each animal receives 5 cc. of a 10% dextrose solution through subcutaneous injection every 6 hours. Under these conditions normal animals may be relied upon to work continuously with only a small decrease, if any, in rate of energy output for a minimum of 5 days. By administration of large amounts of dextrose and some protein we have been able to extend the length of minimum work period to 11 days.

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[1] Heron, W. T., Ingle, D. J., and Hales, W. M., *Science*, 1932, **76**, 550.

Of 35 adrenalectomized animals kept under optimum living conditions 32 died in an average time of 15 days with a range of 4 to 30 days. For 30 adrenalectomized animals which were worked immediately following operation complete "fatigue" took place in an average time of 15 hours with a range of 4 to 33 hours. These animals succumbed in an average time of 2 hours following "fatigue". The 3 animals from the first mentioned group which had survived adrenalectomy for an excess of 30 days and who were normal in weight, appearance, and behavior all "fatigued" and died within 20 hours after beginning work. From this observation and the additional one that control animals subjected to severe sham operations work and survive as normals we regard the effect of operation itself as of small consequence. It was definitely established that maintenance of anesthesia was a most important factor along with work performance in bringing about this rapid manifestation of adrenal insufficiency.

Administration of an adrenal cortex extract prepared by E. C. Kendall* gave striking results. Small doses were effective in prolonging the survival period and increasing the amount of work done. It was possible to bring about temporary recovery from complete "fatigue". Large doses (½ cc. every 6 hours) given to an animal whose work period began immediately following operation prolonged the period of muscular contraction to 145 hours and the survival period to 147 hours. Although the work done was somewhat less than that accomplished by any normal animal, this survival period compares favorably with that expected of a normal animal under these conditions. With further experimentation on dosage we hope to evaluate adequately the capacity of such cortex hormone preparations to maintain a normal energy output in adrenalectomized rats.

The writer has had consistent success in making autoplastic grafts of the adrenals to ovaries in females and he has been only slightly less successful with similar transplants to the spleen in males. These animals gain weight normally and survive indefinitely while their controls died in each case within 30 days. Most significant is the finding that they work normally. Histological examination of the grafts reveals normal cortex tissue. Adequate evaluation of the condition of the medulla has not yet been made but the indications are that it has degenerated. Since it is highly improbable that such grafts would reestablish nerve connections, it appears that nervous innervation is not necessary for a normal secretory activity of the cortex. Also, since the medulla has been shown to be non-functional when denervated we assume that it could have played no part in maintaining the normality of these animals.

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* It is a pleasure to acknowledge my indebtedness to Doctor Kendall for the material supplied to me in this work.

7042 C

Relation of Resistance and Allergy in Tuberculosis as Shown by Vaccinating Rabbits with B. C. G.

B. J. CLAWSON.

From the Department of Pathology, University of Minnesota.

Resistance. Resistance to a subsequent inoculation of a virulent bovine strain of the tubercle bacillus was developed by vaccinating with B. C. G. Four rabbits were inoculated at 4 weekly intervals with 1 mg. of B. C. G. in 1 cc. of salt solution in each of the following methods: (1) living organisms, subcutaneously; (2) living organisms, intravenously; (3) heat killed (60° C. for 30 minutes) organisms, subcutaneously; and (4) heat killed organisms, intravenously.

The basis of the 4 methods of administering the vaccine was suggested by previous experiments with streptococcic vaccination in which it was found that the intravenous method of giving the vaccine was superior to the subcutaneous method in developing resistance and in not producing allergy. This was true with both living and heat-killed organisms.

Three weeks after the last injection of the B. C. G. vaccine, 9 nonvaccinated rabbits, 4 rabbits vaccinated intravenously with the timothy grass bacillus, and the 16 rabbits vaccinated with B. C. G. were inoculated intravenously with 0.01 mg. of virulent bovine tubercle bacilli. The length of life following the injection of the virulent strain and the degree of involvement, if tuberculous lesions were present, were compared in the nonvaccinated and vaccinated injected animals.

The 9 nonvaccinated rabbits died with extensive pulmonary and generalized tuberculosis, 27, 30, 31, 36, 44, 54, 71, 76, and 97 days respectively after receiving the lethal dose of the virulent bovine strain. The average length of life in these 9 animals after injection was 52 days.

The 4 animals which were vaccinated with the timothy grass bacillus were used for controls for specificity of any resistance which might develop from the vaccination. Following the inoculation with the bovine bacillus these 4 animals rapidly developed pulmonary and generalized tuberculosis and died 26, 33, 35, and 45 days respectively after the virulent injection. The average length of life after injection was 35 days. No protection was evident from vaccination with this organism. It was even suggested that the vaccination with the timothy grass bacillus might in some way have hastened the course of the infection.

One of the 4 rabbits vaccinated subcutaneously with living B. C. G. died the night following the injection. At necropsy no tuberculosis was found. The animal had a positive Mantoux test. It is possible that death was due to an allergic shock. The remaining 3 animals of this group were in good condition 142 days after the injection at which time they were killed. One had multiple firm tubercles in the lungs and kidneys. The amount of involvement was much less, however, than in the non-vaccinated animals. The remaining 2 animals, when killed, had no lesions. Not including the animal which died on the day of inoculation, it is seen that the average length of life of the rabbits in this group was more than 142 days.

Of the 4 animals vaccinated intravenously with living B. C. G., 2 died with extensive pulmonary tuberculosis 52 and 102 days respectively after being injected with the virulent bovine strain. The third was killed in the 141st day. Many firm tubercles were found in the lungs. The fourth animal was killed at 156 days after injection. There were several firm tubercles in the lungs and kidneys. The degree of involvement was decidedly less than in the nonvaccinated animals. The average length of life in this group was more than 113 days.

One of the 4 animals vaccinated subcutaneously with heat-killed B. C. G. died 85 days from the time of injection with the virulent bovine strain and one at 96 days. Both had extensive pulmonary tuberculosis. Two are living at 108 days but show clinical evidence of tuberculosis. The involvement in the animals which died was as great as in the nonvaccinated animals.

Of the 4 rabbits vaccinated intravenously with heat-killed B. C. G., one died with a nontuberculous bronchopneumonia 111 days after receiving the lethal bovine injection. A few small firm tubercles were found, however, in the lungs and kidneys. The other 3 animals, though in good condition, were killed 141 days after infection. Two had a few firm tubercles in the lungs and kidneys. The degree of involvement was much less than in the nonvaccinated animals. One of the 4 had no gross or microscopic evidence of tuberculosis. The average length of life in this group was more than 141 days.

Allergy. Allergy or hypersensitiveness, indicated by a positive skin reaction following an intradermal injection of 1 mg. of old tuberculin, was studied in animals vaccinated by the above methods. Tests for hypersensitiveness were made on 30 rabbits, 9 of which were included in the experiments on resistance. Of the 30 animals, 5 were vaccinated subcutaneously with living organisms, 10 intravenously with living organisms, 7 subcutaneously with heat-killed organisms, and 8 intravenously with heat-killed organisms.

All of the 5 animals vaccinated subcutaneously with living organisms had a positive skin test 2 to 3 weeks after the last injection of the vaccine. Five of the 10 vaccinated intravenously with living organisms had positive tests for allergy. One of the 7 vaccinated subcutaneously with heat-killed organisms gave a positive test. Allergy was not present in any of the 8 animals which were injected intravenously with heat-killed organisms. The skin tests were repeated in most cases at intervals up to 16 weeks after the last injection of vaccine. A positive test did not appear later in any, if it had not been present at the first testing (2 to 3 weeks after the last injection of the vaccine).

It may be concluded from the greater length of life in the vaccinated than in the nonvaccinated animals, following injection, and from noting the less amount of involvement or absence of infection in the vaccinated animals and the absence of any resistance in the group vaccinated with the timothy grass bacillus, that vaccination with B. C. G., living or heat-killed, gives a marked degree of specific resistance to rabbits against subsequent infection with a virulent bovine strain. This resistance besides the greater length of life was manifested as retarded and fewer lesions and in a few cases in complete absence of lesions. While resistance was present in all of the above 4 groups, it seemed to be greatest in the groups vaccinated subcutaneously with living B. C. G. and intravenously with heat-killed B. C. G. All of the first were allergic, and allergy was not present in any of the second.

The experiments on resistance and allergy seem to show that, while allergy may be an associated phenomenon of resistance, a high degree of resistance against a tuberculous infection may be developed without a measurable amount of allergy.

7043 P

Minute Volume Determinations in Mitral Stenosis During Auricular Fibrillation and when Restored to Normal Rhythm.

ARTHUR C. KERKHOF AND HANS BAUMANN.

From the Medizinische Klinik, Freiburg in Breisgau, Germany, and the Department of Medicine, University of Minnesota, Minneapolis General Hospital.

Lewis,[1] using dogs and cats, was able to demonstrate that the minute output of the heart was decreased about 20% when the auricles were caused to fibrillate by faradic stimulation. He made the observation also that the more rapid the ventricular rate, the greater the decrease in minute volume.

Eyster and Swarthout,[2] in similar experiments on dogs, were able to produce a decrease in minute volume of from 15-79% when the auricles were caused to fibrillate. The ventricular rate in their experiments was increased after fibrillation was induced so that the actual decrease in minute volume cannot be entirely attributed to the fibrillation of the auricles.

Smith, Walker and Alt[3] found that the minute volume increased approximately 30% when normal rhythm was restored by quinidine in cases of auricular fibrillation which had been previously compensated by digitalis. Realizing that in mitral stenosis auricular contraction plays an appreciable rôle in the filling of the left ventricle, we used only mitral stenosis cases in our series. The experiments were carried out as follows: Four cases of mitral stenosis with auricular fibrillation, some of which were decompensated, were treated with digitalis until well compensated and until the ventricular rate was between 60 and 70 per minute. These cases were kept on digitalis and this rate was maintained for some period of time before the actual measurements of minute volume were begun. These were done by the acetylene method as advocated by

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[1] Lewis, *J. Exp. Med.*, 1912, **16**, 395.

[2] Eyster and Swarthout, *Arch. Int. Med.*, 1920, **25**, 317.

[3] Smith, Walker and Alt, *Arch. Int. Med.*, 1930, **45**, 706.

Grollman.[4] Decompensated cases became well compensated under the regime and in these cases, minute volume determinations were not begun until the vital capacity had reached its highest level. Then the minute volume was determined in each case and repeated until the volumes checked. Continuing the digitalis at its maintenance level, quinidinization was then begun. After regular rhythm had been established and the amount of quinidine had been reduced to a maintenance dose, several days were allowed to elapse. Minute volumes were again determined and repeated until satisfactory checks were obtained.

Using this method in 4 cases, we found that the increase in minute volume when regular rhythm was restored was from 22% to 40% with an average of 30.8%.

The following cases will serve as examples:

Case 1. Admitted to the Medizinische Klinik, Freiburg, in Breisgau, Germany, in a state of marked cardiac decompensation. A diagnosis of mitral stenosis with auricular fibrillation was made. She was treated with digitalis and given the usual cardiac regime. After several weeks of this regime, she became compensated and her ventricular rate was held around 60-70 per minute. A few weeks after she became compensated, her minute volume was determined and a value of 2.03 liters per minute was obtained. She was quinidinized and a normal rhythm was established. After a pause of a few days, her minute volume was determined and value of 2.75 liters per minute was obtained. This represented an increase of 35.8%.

Case 2. Admitted to the Minneapolis General Hospital with a diagnosis of mitral stenosis and auricular fibrillation. Her ventricular rate was rapid but there was very little decompensation present. Increased doses of digitalis reduced her ventricular rate to 60-70 per minute and as she was now completely compensated, her minute volume was determined and a value of 2.55 liters per minute was obtained. After the normal rhythm had been established through the use of quinidine and a sufficient period of time had been allowed to elapse, her minute volume was 3.2 liters per minute, or an increase of 25.5%.

Case 3. Admitted to the Minneapolis General Hospital with a diagnosis of mitral stenosis, mild aortic insufficiency, and auricular fibrillation. He had been digitalized before admission but was kept on digitalis alone for a few days. His rate remained around 60-65 per minute. Minute volume determinations were then done, giving a value of 2.41 liters per minute. After regular rhythm was restored with quinidine, his minute volume rose to 3.37 liters per minute, a gain of 40%.

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[4] **Grollman, The Cardiac Output of Man in Health and Disease. Charles C. Thomas, 1932.**

Case 4. Admitted to the Minneapolis General Hospital with mitral stenosis and auricular fibrillation. This patient had already been digitalized and the ventricular rate had already been reduced to around 60-70 per minute. After a few days in the hospital, the minute volume was determined as 2.38 liters per minute. She was quinidinized and the regular rhythm restored. Her minute volume rose to 2.90 liters per minute—a rise of 22%.

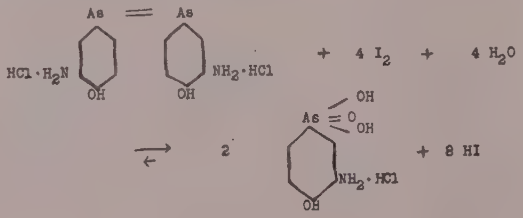
7044 C

A Rapid Method for Quantitative Estimation of Arsenic in Arsphenamine.*

HAROLD N. WRIGHT.

From the Department of Pharmacology, University of Minnesota School of Medicine.

The direct titration of the arsenic in arsphenamine with a standard iodine solution was first used by Gaebel,[1] who also found that the reaction was a reversible one, namely



The titration of inorganic arsenious compounds with iodine is also a reversible reaction, but may be carried to completion, as shown by Washburn and Strachan,[2] by maintaining the pH of the reaction mixture between 9 and 4. Gaebel found, however, that this was not the case with the reaction between arsphenamine and iodine.

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* A part of the funds used in this research were supplied from the Medical Research Fund granted by the Board of Regents of the University of Minnesota.

[1] Gaebel, G. O., *Arch. Pharm.*, 1911, **249**, 241.

[2] Washburn, E. W., and Strachan, E. K., *J. Am. Chem. Soc.*, 1913, **35**, 681.

Myers and Du Mez[3] from a comparative study of several methods of quantitative determination of the arsenic in arsphenamine, rejected Gaebel's titration method because of the low results obtained. Nevertheless, the direct titration of both arsphenamine and neoarsphenamine with iodine has been employed by a number of workers, most of whom have not taken into account the fact that the reaction does not go to completion. The method has been used quite extensively, for instance, for the simultaneous oxidation of arsenic and sulphur in the differential analysis of the composition of neoarsphenamine and sulpharsphenamine. The accuracy of the conclusions drawn from such simultaneous analyses, particularly in regard to the sulphur determinations, depends, of course, upon the absolute accuracy of the values assigned to the arsenic oxidation.

Macallum,[4] quoting 3 analyses of a single sample of arsphenamine, with a 3.5% titration error between samples, and assuming 31.3% arsenic content for the arsphenamine (anhydrous arsphenamine contains 34.2% arsenic; with 1 mol. of CH_3OH 31.85%; with 2 mols. of H_2O 31.57%) derived the relationship of 7.755 mols. (theoretical 8 mols.) of iodine for the titration of arsphenamine with iodine. Elvove,[5] in making differential analyses of the composition of samples of neoarsphenamine and sulpharsphenamine, employed the factor thus derived by Macallum.

It was felt, therefore, that the quantitative determination of sulphur fractions in neoarsphenamine and sulpharsphenamine by the simultaneous oxidation of arsenic and sulphur were of doubtful value, unless it could be shown that the reaction between the iodine and arsenic attained a constant point of equilibrium under the conditions of the titration, and the equilibrium point of the reaction was accurately determined. Under such circumstances the direct titration of arsphenamine with iodine would also constitute a far more rapid method for the determination of the arsenic content of samples of arsphenamine than any of those in present use.

A comparative study of the results obtained by the direct titration method with those obtained by the gravimetric method was, therefore, undertaken. The results obtained confirm Gaebel's statement that the reaction between arsphenamine and iodine is incomplete, and cannot be carried to completion by the addition of mild alkalies such as sodium bicarbonate, borate or acetate. Friedman[6] has shown that the reaction becomes progressively less complete in strongly acid solutions. We have found the titration satisfactory provided the initial pH is not below 2, the approximate pH of the di-hydrochloride of arsphenamine.

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[3] Myers, C. N., and Du Mez, A. G., *U. S. Pub. Health Rep.*, 1918, **33**, 1003.

[4] Macallum, A. D., *J. Am. Chem. Soc.*, 1922, **44**, 2578.

[5] Elvove, E., *U. S. Pub. Health Rep.*, 1924, **39**, 750.

[6] Friedman, L., *J. Lab. and Clin. Med.*, 1925, **11**, 528.

TABLE I.
Comparative Analysis of Arsphenamine by Gravimetric and Iodine Titration Methods.

Sample	% As (Grav.)	% As (I_2titr.)	Mols. I_2	Ratio G/I_2
A_1	31.57	29.81	7.555	1.059
A_2	31.65	29.84	7.543	1.061
B_1	31.17	29.55	7.584	1.055
B_2	31.31	29.54	7.548	1.060
C_1	31.55	29.70	7.532	1.062
C_2	31.40	29.67	7.560	1.058

Number of analyses to date—12.

We are able to conclude, therefore, that unless the solution is strongly acid, the reaction between arsphenamine and iodine reaches a point of equilibrium when 7.55 ± 0.03 mols. of iodine (theoretical 8.0) have been used. Consequently, by means of the conversion factor $8.0/7.55 = 1.060$ we can convert the amount of iodine actually used in a given titration into the amount which would be used theoretically if the reaction could be carried to completion, from which figure the percentage of arsenic in the sample is calculated.

The method is extremely rapid, the end point is sharp (unless the solution is too acid) using starch as an indicator, and the conversion factor has been found to be applicable to all samples of arsphenamine so far tested. Samples of arsphenamine contaminated with oxidizable sulphur compounds would, of course, give too high values for the arsenic content.

7045 P

Relation Between Colloidal Properties and Toxicity of Arsphenamine and Neoarsphenamine.*

A. BIEDERMANN, E. HANSSEN AND HAROLD N. WRIGHT.

From the Department of Pharmacology, University of Minnesota Medical School.

Bauer,[1] Klemensiewicz,[2] Sherndal,[3] Raiziss and Gavron,[4] and others, have demonstrated that both arsphenamine and neoarsphenamine belong to the group of emulsoid "semi-colloids". Hirschfelder and Wright[5] studied the effects of neoarsphenamine and other semi-colloids on solutions of egg albumin and rabbit plasma and concluded that "the fact that most of these substances react strongly with proteins, producing ultramicroscopic, and in some cases macroscopic, changes in the proteins, lends strong support to the idea that anaphylactoid and febrile reactions following the injection of these substances into the blood stream, are due to definite changes brought about in the hydration and aggregation of the blood proteins".

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* A part of the funds used in this research were supplied by a grant from E. R. Squibb & Sons, New York; a further portion was supplied from the Medical Research Fund granted by the Board of Regents of the University of Minnesota.

Raiziss and Gavron determined the degree of colloidality of a few samples of arsphenamine and neoarsphenamine by means of dialysis, using parchment membranes. Such membranes are relatively impermeable, and permitted only a small fraction of the arsenical to pass through.

Dialysis experiments using both arsphenamine and neoarsphenamine with viscose and cellophane membranes of varied permeability indicate that both of these arsenicals contain particles of many sizes ranging from the true crystalloid which will pass through membranes of slight permeability to particles which must be large aggregates since they fail to pass through the most permeable membranes we have employed.

Arsphenamine HCl may be maintained in aqueous solution under nitrogen indefinitely without evidence of deterioration, since the iodine value remains constant and tests for arsenoxide were always negative. Dialysis through viscose membranes does not produce either oxidation or precipitation. Solutions of sodium arsphenamine under nitrogen are stable in the absence of a membrane. When a membrane is interposed sodium diffuses out and the arsenical precipitates within the membrane. Attempts to stabilize the solutions by dialyzing against buffered solutions containing sodium were unsuccessful. Solutions of sodium arsphenamine are, however, perfectly stable when dialyzed against a 1:10,000 solution of sodium formaldehyde sulphoxylate. Solutions of neoarsphenamine likewise develop an acid reaction, and undergo hydrolysis, oxidation

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[1] Bauer, H., Chem. Abst. George Speyer Hauses, 1919, Gustave Fischer, Jena.

[2] Klemensiewicz, M. Z., *Bull. soc. chim. de France*, 1920, **27**, 820.

[3] Sherndal, A. E., *J. Lab. and Clin. Med.*, 1933, **7**, 723.

[4] Raiziss, G. W., and Gavron, J. L., *J. Pharmacol. and Exp. Therap.*, 1922, **20**, 163.

[5] Hirschfelder, A. D., and Wright, H. N., *J. Pharmacol. and Exp. Therap.*, 1930, **39**, 13.

and precipitation unless dialyzed against a very dilute solution of sodium formaldehyde sulphoxylate.

Sodium formaldehyde sulphoxylate when added in very dilute concentrations to solutions of arsphenamine HCl, sodium arsphenamine or neoarsphenamine acts as a powerful peptizing agent, markedly increasing the amount of the crystalloid fraction and decreasing the toxicity of the drug.

The crystalloid and colloid fractions of the arsenicals were obtained by dialysis through viscose sausage skin membranes (Visking Corporation, Chicago) or cellophane sheets (Dupont No. 600). Sodium arsphenamine and neoarsphenamine were dissolved in and dialyzed against dilute solutions of sodium formaldehyde sulphoxylate adjusted to pH 9.0. Water solutions of arsphenamine HCl were used. The dialysate was removed at 24 hour intervals and replaced by fresh solution. The amount of arsenical passing through the membrane decreased rapidly in each succeeding period, reaching an equilibrium in 4 to 5 days, when no more arsenical passed through the membrane. The first 24 hour dialysate, kept under nitrogen 5 days was used as the crystalloid fraction. The arsenical remaining in the dialyzing sac after 6 days dialysis was used as the colloid fraction. Toxicity studies on the whole drug were made with solutions maintained under nitrogen for 6 days. Freshly boiled distilled water, cooled under nitrogen was used. Arsphenamine solutions were alkalinized under nitrogen. All solutions were tested for arsenoxide by Rosenthal's[6] method and found negative.

The results obtained are shown in Table I.

TABLE I.
Comparative Toxicity of Crystalloid and Colloid Fractions of the Arsphenamines.

Drug	Fraction	No. Rats	Immediate deaths		Delayed deaths	
			mg./K	% deaths	mg./K	% deaths
Arsphenamine HCL	Whole drug	30			35	25
" "	Crystalloid	34			60	50
" "	Colloid	48	25	100	20	50
Sodium Arsphena-	Whole drug	32			125	75
" mine	Crystalloid	29			125	75
" "	Colloid	13	100	none		
Neoarsphenamine	Whole drug	277			375	50
"	Crystalloid	87			450	50
"	Colloid	56	35	50		

In most of the experiments the toxicity of the crystalloid fraction was less than that of the whole drug. Rats injected with fatal doses of the crystalloid fraction invariably died several days after the injection, as did those receiving the whole drug, with typical symptoms of arsenical poisoning.

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[6] Rosenthal, S. M., *U. S. Pub. Health Rep.*, 1932, **47**, 933.

Rats injected with fatal doses of the colloid fraction showed a reaction immediately after injection. The animals developed severe dyspnea, and in many cases prolonged apnea; there were clonic convulsions of the hind legs; in most cases there was a bloody exudate from the nose and mouth. Many animals died within 15 minutes, almost all within 3 hours. Animals living longer than 3 hours usually recovered.

These experiments indicate that it is the colloid fraction of these arsenicals which is responsible for the immediate toxic reactions, whereas the crystalloid fraction is less toxic than the whole drug and produces only delayed symptoms of arsenical poisoning when injected in toxic amounts.

The authors desire to express their deep appreciation to Dr. A. D. Hirschfelder for his valuable suggestions and constant interest in the progress of the work.

7046 P

Therapeutic Efficiency of Arsphenamine and Neoarsphenamine Fractions.*

E. HANSSEN AND HAROLD N. WRIGHT.

From the Department of Pharmacology, University of Minnesota, School of Medicine.

Biedermann, Hanssen and Wright[1] have shown that arsphenamine hydrochloride, sodium arsphenamine or neoarsphenamine may be separated by means of dialysis through viscose membranes into 2 fractions, the one consisting of particles which readily pass through the membrane (crystalloid fraction), the other consisting of particles which fail to pass through the membrane after repeated dialysis until no further arsenical passes through the membrane (colloid fraction). The technic and precautions used are similar to those described in the previous paper. In all experiments with sodium arsphenamine and neoarsphenamine it was found necessary to use 1/10,000 sodium formaldehyde sulphoxylate as a stabilizing agent.

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* A part of the funds used in this research were supplied from the Medical Research Fund granted by the Board of Regents of the University of Minnesota.

[1] Biedermann, A., Hanssen, E., and Wright, H. N., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 172.

The previously reported study having shown that the 2 fractions of these semi-colloid arsenicals possessed markedly different properties in regard to toxicity, the present study was undertaken to determine whether or not these separated fractions possessed any differences in therapeutic activity.

The experiments on the therapeutic properties of the various arsenicals and their fractions were carried out on albino rats of the Wistar Institute strain, weighing 150-200 gm., and inoculated 24 hours before injection of the arsenical with approximately 300,000 organisms of *Trypanosoma equiperdum.*† Control animals were included in every experiment and these animals invariably died in 3 to 5 days. Test animals were not recorded as cured unless repeated microscopic examinations of the blood were negative for trypanosomes for 30 days after injection of the arsenical.

TABLE I.

Comparison of Separation of Neoarsphenamine Fractions with their Therapeutic Activity.

Sample	Dialysis			Therapeutic Activity		
	Colloid	Crystalloid	Ratio	Colloid	Crystalloid	Ratio
A_1	220 mg.	48 mg.	4.6	24 mg./Kg.	3 mg./Kg.	8.0
A_2	401 "	108 "	3.7	36 " "	7 " "	5.1
A_3	357 "	257 "	1.4	33 " "	8 " "	4.1
B_1	246 "	220 "	1.1	16 " "	9 " "	1.8

No. Experiments = 40
No. animals used = 800 approx.

The results clearly indicate that the crystalloid fraction of neoarsphenamine has a much higher therapeutic effect than that of the colloid fraction. In general, we have found that the smaller the amount of the crystalloid sample (first 24 hour dialysate) the higher the therapeutic efficiency. Thus in Sample A_1 above, the viscose membrane was relatively impermeable and the crystalloid fraction was found to have a very high therapeutic activity, only 3 mg./kg. being required as against 24 mg./kg. for the colloid fraction. In another experiment using the same brand and lot number but a very porous membrane the crystalloid fraction contained 323 mg., the colloid fraction 233 mg., ratio 0.7, and the therapeutic activity of the crystalloid fraction was found to be 11 mg./kg., and 13 mg./kg. for the colloid fraction, giving a ratio of only 1.2 in favor of the crystalloid fraction, a result agreeing well with the poor separation obtained.

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† We wish to express our sincere thanks to Dr. A. L. Tatum, Department of Pharmacology, University of Wisconsin, who kindly furnished us with animals inoculated with the organism.

We have carried out a small number of similar experiments with sodium arsphenamine and find that the results obtained are qualitatively similar. In one experiment, for instance, the crystalloid fraction was found to be curative in doses of 2 mg./kg. while 7 mg./kg. of the colloid fraction were required.

The authors desire to express their deep appreciation to Dr. A. D. Hirschfelder for his valuable suggestions and constant interest in the progress of the work.

Illinois Section.

University of Chicago, October 10, 1933.

7047 P

Mechanism of Pancreatic Secretion.

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From the Department of Surgery, University of Chicago.

In a series of experiments performed on cats anesthetized by urethane, Mellanby[1] found that the introduction of bile of the proper reaction into the duodenum caused a copious secretion of pancreatic juice. The activity of the bile was found to be due to cholic acid and its compounds. The interpretation suggested was that the bile salts in the duodenum become associated with and make possible the absorption of secretin into the portal blood, and that this event rather than the passage of acid from the stomach is responsible for pancreatic secretion. It would seem to follow, if this theory were true, that the removal of bile from the intestine would check or greatly decrease the secretion of pancreatic juice. To determine this point, pancreatic and biliary fistulae were prepared in each of 2 dogs. The method has been previously described (Dragstedt, Montgomery, and Ellis[2]), and permits the quantitative collection of each secretion during an entire 24 hour period or for several days. In the first test animal I secreted 4609 cc. of pancreatic juice and 692 cc. of bile in 6 days, when practically all of the bile and pancreatic juice was returned to the alimentary tract. During the succeeding 6 days no bile was returned and 5998 cc. of pancreatic juice and 416 cc. of bile were secreted. In the second test 3971 cc. of pancreatic juice and 752 cc. of bile were secreted in 6 days when both secretions were returned to the stomach whereas in the succeeding 6 days when only the pancreatic juice was returned, 4688 cc. of pancreatic juice and 426 cc. of bile were secreted. The results obtained with animal 2 were essentially similar. In each case the dog secreted a little more pancreatic juice when no bile was present in the intestine than when it was returned by mouth.

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[1] Mellanby, J., *J. Physiol.*, 1925, **60**, 85; 1926, **61**, 419, 489; 1928, **66**, I, **64**, 331.

[2] Dragstedt, L. R., Montgomery, M. L., and Ellis, J. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 109.

It seems quite definite, therefore, that, in the dog at least, bile salts cannot be considered essential either for the activation of pancreatic secretin or for its passage into the blood in effective form.

7048 C

Removal of Bromsulphalein from the Blood Stream by the Reticulo-Endothelial System.

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The early work of Aschoff, Ribbert, Goldmann and Kiyono showed that colloidal dyes are removed from the blood stream by the reticulo-endothelial system. Phenoltetrachlorphthalein, a colloidal dye, was shown by Schellong and Eisler[1] to remain longer than normally in the blood stream of rabbits following India ink injection or splenectomy and Saxl and Donath[2] obtained similar results after electrocollargol injections in humans. Merklen, Wolf and Arnovljevitch[3] and Fiessinger and Longchampt[4] believed this dye to be a test of the reticulo-endothelial system rather than of the liver.

Bromsulphalein, introduced by Rosenthal and White,[5] to estimate liver function is supposed to circulate as a soluble dye and to be more specifically excreted by the liver than phenoltetrachlorphthalein. Herlitz,[6] however, on the basis of clinical investigations, called attention to the possibility that bromsulphalein might be excreted through the reticulo-endothelial system in general and Kupffer cells in particular rather than through the hepatic parenchyma. To investigate this we performed bromsulphalein tests on dogs which had been splenectomized or had received India ink injections.

Control dye tests were done on 11 apparently normal animals.

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[1] Schellong and Eisler, *Z. f. d. ges. exp. Med.*, 1928, **58**, 738.

[2] Saxl and Donath, *Wien. Arch. f. i. Med.*, 1927, **13**, 7.

[3] Merklen, Wolf and Arnovljevitch, *Bull. et Mem. de la Soc. Med. des Hop.*, 1925, **49**, 1180.

[4] Fiessinger and Longchampt, *Presse Med.*, 1925, **33**, 1873.

[5] Rosenthal and White, *J. Pharm. Exp. Therap.*, 1924, **24**, 265.

[6] Herlitz, *Acta. Paediat.*, 1931, **12**, 1 (Supp. 5).

Two milligrams of bromsulphalein per kilo body weight was injected intravenously and blood samples taken 5 and 30 minutes later. The percentage of dye in the serum was estimated as outlined by Rosenthal and White.[7] Six of the animals were splenectomized under nembutal anesthesia and one was laparotomized as a control. Bromsulphalein tests were then done on the second or third postoperative day and repeated at various intervals thereafter. The control dog showed no change in the percentage of bromsulphalein in the blood, while the splenectomized animals showed slight increases at the end of 5 minutes. This effect lasted for 3 to 4 weeks, at the end of which time the results were normal. Table I summarizes the results obtained.

TABLE I.
Effect of Splenectomy on Dye Test.

	% 5 min.	% 30 min.
Control (6 dogs)	0-35	0
2-3 days after splenectomy	20-70	0-5
10 " " "	25-60	0
20 " " "	10-25	0
30 " " "	5-20	0

Splenectomy, therefore, causes a slight but nevertheless definite slowing of the removal of bromsulphalein from the blood.

Each of the other 4 animals received an intravenous injection of 50 cc. of an 8% India ink suspension in normal saline. One-half hour later a bromsulphalein test was performed. Two of the dogs were then sacrificed and histological examination made of the liver and spleen. The Kupffer cells were found to be well filled with ink, while the splenic endothelial cells were not so well blocked. The hepatic parenchyma appeared normal in both animals. On the other 2 dogs bromsulphalein tests were done on successive days. Table II summarizes the results.

There was a considerable retention of the dye following reticulo-endothelial blockade with India ink, lasting 48 hours, gradually diminishing so that at the end of 3 to 4 days the elimination becomes normal. The question arises whether this dye retention is due to the actual mechanical stuffing of the reticulo-endothelial cells with ink or to some other factor. Victor, Van Buren and Smith[8] presented evidence to show that the retention of brilliant vital red in the blood stream after ink injections was not due to the carbon particles in the ink but to some other ingredient. The injection of

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[7] Rosenthal and White, *J. Am. Med. Assn.*, 1925, **84**, 1112.

[8] Victor, Van Buren and Smith, *J. Exp. Med.*, 1930, **51**, 531.

TABLE II.
Effect of Reticulo-Endothelial Blockade on Dye Test.

	% 5 min.	% 30 min.
Control (4 dogs)	5-10	0
30 min. after ink injection	70-100	25-50
24 hrs. " " " (2 dogs)	65-100	35-50
48 " " " " (1 ")	90	45
68 " " " "	35	10
92 " " " "	15	0
120 " " " "	20	0

ground graphite suspended in acacia did not impair the ability of the liver to excrete the dye. They thought that the effect of the ink injection was produced through a hindrance in the excretion of the dye into the bile. If this is correct, splenectomy then should have no effect on the dye excretion since the spleen does not have any known rôle in bile formation. Shellong and Eisler,[1] however, obtained almost as much retention of phenoltetrachlorphthalein after splenectomy as after ink injection. We also note a slight effect of splenectomy on bromsulphalein excretion.

Our results do not agree with Rosenthal and Lillie,[9] who found no effect on the bromsulphalein excretion after splenectomy or colloidal quartz injection in rabbits. Our findings support the opinion of Herlitz that bromsulphalein is excreted through the reticulo-endothelial system, the Kupffer cell component of which plays a very important rôle.

7049 C

Specificity of Toxin of a Non-Hemolytic Variant of a Scarlet Fever Streptococcus.

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A strain of hemolytic streptococcus, No. 1452, isolated by Jones and Little[1] from the infected udder of a cow the milk of which had caused an epidemic of scarlet fever was sent to us about 3 years ago. On receipt, this streptococcus formed large mucoid colonies resembling *Str. epidemicus* on ascites blood agar and capsules were demonstrable in India ink preparations. The toxin of this strain corresponded to the specific toxin of the streptococcus of scarlet fever and not to the toxin of septic sore throat.[2]

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[9] Rosenthal and Lillie, *Am. J. Physiol.*, 1931, **97**, 131.

The culture had been kept in the refrigerator on 0.5% blood infusion agar slants. At one transplanting it was noted that the strain had lost its hemolytic and mucoid characteristics; the colonies had become small and biconvex and produced green pigment on ascites blood agar. Stained organisms showed rather short chains of Gram positive cocci, typical of streptococci.

A single mouse passage was made and from the heart's blood 2 types of colony were grown; one, green pigment-producing and non-hemolytic like the parent variant and the other identical with the original hemolytic capsule-producing, mucoid colony-forming organism. In an hemolysin test in blood broth the green producing streptococcus completely failed to lyse the blood cells.

A toxin was prepared from bacteria from each type of colony in meat infusion broth (pH 7.4, 1% peptone, 1% NaCl) by incubating 7 days at 35°C. A 1:1000 dilution of the toxin of the non-hemolytic variant in physiologic saline and a 1:1500 dilution of the toxin of the hemolytic strain gave skin reactions practically equivalent in size and intensity to those produced by the Dick skin test toxin. Generally, however, the toxin of the hemolytic strain produced a slightly larger reaction. Intradermal skin tests with 0.1 cc. of each of the diluted toxins and 0.1 cc. of Dick skin test toxin as control were made on 10 previously determined Dick positive subjects, 6 adults and 4 children. All gave red areas from 1.5 to 2 cm. in diameter with both toxins.

These subjects were then immunized with 5 doses of the Dick toxin. Six weeks after the last dose identical skin tests were made. Nine out of 10 were negative to all 3 toxins. One child remained positive to the Dick skin test toxin and the toxin of the hemolytic strain in a dilution of 1:1000. There was no skin reaction to the toxin of the non-hemolytic variant in a dilution of 1:1000. Five additional Dick positive subjects who had given positive reactions to our toxins both in a dilution of 1:1000, after similar immunization gave negative skin tests to the toxin of the non-hemolytic variant 1:1000 and the toxin of the hemolytic strain 1:1500.

Eight out of 10 Dick negative subjects gave no skin reactions to the same toxins in the same dilution. In a dilution of 1:2000 of both toxins the 9th gave negative results but the 10th continued to be faintly positive to both.

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[1] Jones and Little, *J. Exp. Med.*, 1928, **47**, 957.

[2] Pilot, I., and Davis, D. J., *J. Inf. Dis.*, 1933, **53**, 29.

Specific scarlet fever horse antiserum, 1 :50, neutralized both toxins, 1 :1000, in intradermal tests made before immunization in 3 of the 4 children mentioned above. The 4th child was serum sensitive. This same horse antiserum (1 :50) failed to neutralize the toxin of a septic sore throat strain of *Str. epidemicus* (1 :500) in 5 children who had shown positive cutaneous reactions to sore throat toxin. Nor did it neutralize the toxin of an erysipelas strain of hemolytic streptococcus of the mucoid variety (1 :500) in intradermal tests in one child who had responded positively to the erysipelas toxin.

In this work we add another variation in cultural characteristics of scarlet fever streptococcus. Previous reports indicate that scarlet fever streptococci may or may not ferment mannitol and still produce a specific toxin. Similarly scarlet fever streptococci may become mucoid and encapsulated and still produce specific toxin. In the present work specificity of toxin is retained although the strain had lost its hemolytic property.

7050 P

Loss of Pupillary Light Reflex Resulting from Lesions in the Region of the Posterior Commissure.

H. W. MAGOUN AND S. W. RANSON.

From Northwestern University Medical School.

Systematic exploration of the interior of the brain in the region of transition between midbrain and forebrain has enabled us to trace the central path for the pupillo-constrictor reflex in response to light. This was done with a fine needle electrode accurately placed with the aid of the Horsley-Clarke stereotaxic instrument and the points stimulated were checked by subsequent microscopic examination. These experiments, which have been fully described elsewhere,[1] showed that the impulses responsible for pupillary constriction travel from the optic tract through the superior quadrigeminal brachium and pretectal region but not through the superior colliculus. There appears to be a partial crossing in the posterior commissure and another ventral to the cerebral aqueduct.

Taking advantage of the precise information thus obtained we have been able to obliterate completely and permanently the pupillary light reflex in both eyes by placing small electrolytic lesions in the pretectal region on each side of the posterior commissure. This was done in cats with the aid of the stereotaxic instrument. In these cats both pupils remained for weeks widely dilated and entirely unresponsive to light. That the sphincter nucleus of the third nerve was not damaged was shown by the fact that pupillary constriction still accompanied winking, the well-known lid closure reflex.

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[1] Ranson, S. W., and Magoun, H. W., *Arch. Neurol. and Psychiat.*, in press.

Four of these cats with complete loss of the pupillary light reflex are being allowed to live for a longer period of time in order to make sure that the loss is permanent. Three others were sacrificed after periods ranging from 3 to 7 weeks. Microscopical sections of the brains of these 3 cats showed that the lesions had been accurately placed on both sides of the posterior commissure in the pretectal region and that except for a small area near the midline in the most rostral part of the tectum the superior colliculi were intact. The superior quadrigeminal brachia, the lateral geniculate bodies and optic tracts had not been damaged.

These experiments confirm the conclusions drawn from the stimulation experiments that the pupillary light reflex is mediated through the pretectal region and not through the superior colliculus. The slight amount of damage to the rostral part of the superior colliculus we regard as inconsequential. But in order to remove any possible objection on this ground other experiments are in progress in which the effort is being made to abolish the pupillary reflex with still smaller lesions which do not in any way involve the superior colliculi.

7051 C

Changes in the T Wave of the Electrocardiogram in Acute Rheumatic Fever.*

LEO KOHN. (Introduced by Louis N. Katz.)

From the Heart Station, Michael Reese Hospital, Chicago.

Transitory flattening or inversion of the T wave of the electrocardiogram has been found during the active stage of rheumatic fever by Rothschild, Sacks and Libman,[1] by Shapiro,[2] and by Master.[3]

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* Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

[1] Rothschild, M. A., Sacks, B., and Libman, E., *Am. Heart J.*, 1927, **2**, 356.

[2] Shapiro, M. J., *Am. Heart J.*, 1930, **5**, 504.

[3] Master, A. M., *Am. J. Med. Sci.*, 1931, **181**, 211.

However, Schwartz and Weiss[4] have claimed that the T wave of children with acute rheumatic fever does not differ from the T wave of the normal child. In view of this difference of opinion, it was decided to reinvestigate this subject.

Electrocardiograms of children suffering from rheumatic fever were selected for this study from the 8300 records in the electrocardiographic files of the Heart Station of Michael Reese Hospital. From these were selected those in which 2 or more electrocardiograms had been taken. The clinical charts of these patients were studied, and 12 cases, with electrocardiograms in both the active and inactive periods of the disease, were selected. The rheumatic fever was assumed to be active when a majority of the following signs and symptoms was found: rapid heart rate, pyrexia, "sore throat", joint pains, acute pericarditis and an elevated white blood count.

The electrocardiographic changes found in these cases confirm the observations of Cohn and Swift,[5] Rothschild, Sacks and Libman,[1] Shapiro,[2] and Master,[3] *i. e.*, the P wave was often altered in size, shape and direction; the P-R interval was often lengthened; the QRS complex was often notched, lengthened, or changed in ampli-

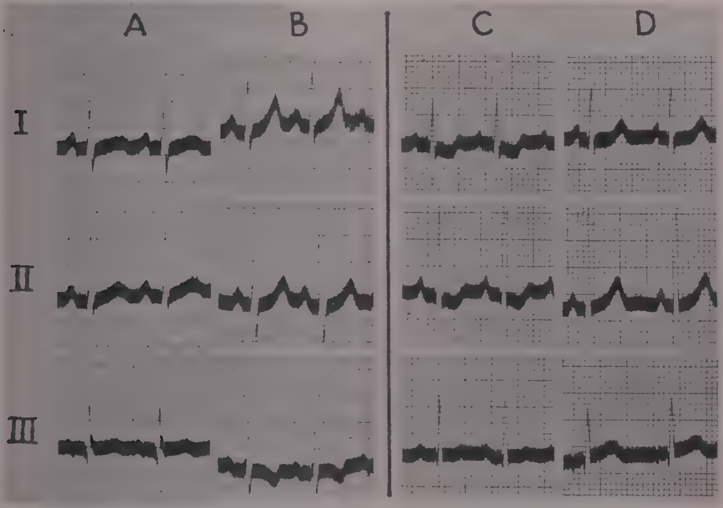

FIG. 1.
Segments of the electrocardiogram, standard 3 leads, taken during the active and inactive stages of acute rheumatic fever in 2 patients. A, active stage of one patient; B, inactive stage of same patient; C, active stage of a second patient; D, inactive stage of second patient.

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[4] Schwartz, S. P., and Weiss, M. M., *Am. J. Dis. Child.*, 1929, **38**, 699.

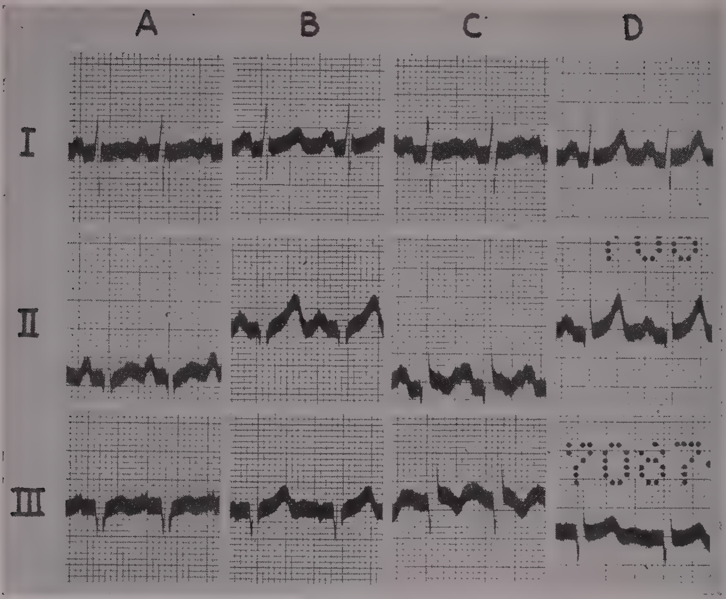

FIG. 2.

Segments of the electrocardiogram, standard 3 leads, taken during the active and inactive stage of acute rheumatic fever. A and C, during 2 active periods; B and D, during 2 inactive periods. Segments arranged in chronological order.

tude or direction; the S-T segment was often anisoelectric or sloping; and the T wave was flattened, diphasic, isoelectric or inverted. With recovery from the active stage of the disease there was a tendency, after a varying period of time, for the electrocardiogram to return towards normal. Typical changes are shown in Figs. 1 and 2.

The T wave during the active phase not only had become small but usually had lost its normal peaked top (Fig. 1, Record A). When the T wave was inverted, it tended to assume the appearance of the coronary T wave (Fig. 2, Record C, Lead III). With recovery from the active stage of rheumatic fever, the T wave tended to become taller and sharply peaked (Figs. 1 and 2. Records B and D). Some cases were followed through successive active episodes and intervening inactive periods, and the same changes were found with each new cycle of the disease (Fig. 2). T wave changes similar to those described in acute rheumatic fever, but more transitory, have been described in pneumonia (Master, Romanoff and Jaffe[6]; Arnett and Harris[7]; Abt and Vinnecour[8]), in typhoid fever (Chagas[9]), and in scarlet fever (Shookhoff and Taran[10]).

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[5] Cohn, A. E., and Swift, H. F., *J. Exp. Med.*, 1924, **39**, 1.

Digitalis may produce changes in the S-T segment and T wave similar to those found in acute rheumatic fever (Cohn, Fraser and Jamieson[11]), but digitalis was used in only a few of the cases here reported. The changes are therefore most likely due to the rheumatic infection itself.

Summary. A study was made of the electrocardiograms of 12 cases of rheumatic fever in children, taken during both the active and inactive stages of the disease. During the active stages, the T wave was softly rounded and small, sometimes isoelectric, diphasic or inverted, and during recovery the T wave became upright, tall, and sharply peaked.

I am grateful to Dr. Louis N. Katz for his advice and suggestions.

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[6] Master, A. M., Romanoff, A., and Jaffe, H., *Am. Heart J.*, 1931, **6**, 696.

[7] Arnett, J. H., and Harris, S. E., *Med. Clinics of N. America*, 1931, **15**, 503.

[8] Abt, A. F., and Vinnecour, M. I., in press.

[9] Chagas, E., *C. R. de Soc. Biol.*, 1930, **106**, 505.

[10] Shookhoff, C., and Taran, L. M., *Am. J. Dis. Child.*, 1931, **42**, 554.

[11] Cohn, A. E., Fraser, F. R., and Jamieson, R. A., *J. Exp. Med.*, 1915, **21**, 593.

Missouri Section.

Washington University Medical School, October 11, 1933.

7052

Preparation of Gonadotropic Substance by Tungstic Acid Precipitation.

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We presented data on the quantitative precipitation of small quantities of prolan with tungstic acid.[1] Extending our work to pregnancy urine we found that tungstic acid would produce a precipitate which contained most of the gonadotropic substance and a considerable quantity of both protein and tungstic acid. Since our interest lay in the purification of the gonadotropic material, several processes for the removal of the impurities were studied. The barium precipitation used in our earlier work and which was also used for the decomposition of the phosphotungstate precipitate (Wiesner and Marshall,[2] Zondek, Scheibler and Krabbe[3] proved to be less satisfactory than the treatment with certain inexpensive alkaloids.

To one liter of urine add 20 cc. of sodium tungstate (10%). Acidify this mixture to congo red paper with sulfuric acid. Centrifuge and wash the precipitate once with acetone. A little distilled water and some solid brucine are added, the mixture thoroughly stirred and then centrifuged. The clear supernatant solution contains nearly all of the gonadotropic substance of the original urine.

Although our experience is somewhat limited it seems probable that sodium molybdate may be used instead of sodium tungstate. Quinine and possibly benzidine hydrochloride or 8, hydroxy-quinoline may be used in place of the brucine. Barium hydroxide gives less satisfactory preparations and sodium hydroxide or aqueous pyridine dissolves nearly all of the tungstate precipitate.

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[1] Katzman, P. A., and Doisy, E. A., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1188.

[2] Wiesner, B. P., and Marshall, P. G., *Quart. J. Exp. Physiol.*, 1931, **21**, 147.

[3] Zondek, B., Scheibler, H., and Krabbe, W., *Biochem. Z.*, 1933, **258**, 102.

Since we have found that some purified preparations of the gonadotropic substance fail to give a precipitate with tungstic acid, it is probable that the active material is carried down by adsorption. The function of the brucine is 2-fold; to release the gonadotropic material from the precipitate and to form an insoluble tungstate which prevents the tungsten from dissolving. As shown in the table, the tungstic acid procedure gives much higher yields than those obtained by the benzoic acid method (Katzman and Doisy[4]). The yields obtained by the use of brucine or quinine compare favorably with those of the crude sodium hydroxide solutions of the tungstate precipitate.

TABLE I.
Gonadotropic Substance Obtained from Urine by Different Procedures. Rat Units per Liter of Urine.

Urine No.	Benzoic Acid Method	Tungstic Acid with NaOH	Tungstic Acid with Brucine	Tungstic Acid with Quinine
118	<160	>4430 <5175	8300	>4500
119	170	6000	>5940 <7180	>4690
120	<460	5500	>6600 <7800	>3300 <4980
121	>1000 <1200	>2750 <4150	2970	<2500
122	>1050 <1360	>5840 <7700	>6380 <7000	>6800
123	>1200 <1500	>4600 <5940	5810	5810
124	1750	7100	6640	
125	>700 <1000	6600	6500	
126	>570 <1140	3819	>2750 <4150	
127	<900	>3300 <4400	>4150 <5500	
128	850	5540	>4950*	

*Using hydroxy-quinoline instead of brucine >2575 <4300 R. U.

The small amount of brucine present in these extracts may be removed by extraction with chloroform after the addition of dilute sodium hydroxide to ensure that the alkaloid is present in the free form.

The data on the tungstic acid procedure given in the table were obtained with 500-1000 cc. samples of urine, while the yields by the benzoic acid method are based on results from 100-200 liter batches. Preliminary experiments indicate that the new process will work satisfactorily on large volumes of urine.

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[4] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

7053 P

Skin Sensitivity and Antibody Production upon Administration of Normal and Immune Serum in Rabbits.

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In studies upon the antibody response in human subjects following administration of serum, Tuft and Ramsdell[1, 2] have noted that normal serum is weakly antigenic and immune serum highly antigenic, as indicated in tests made for precipitins, skin sensitivity, anaphylactic antibodies and transferable human and guinea pig skin sensitizing antibodies. Administration of normal horse serum was followed by serum sickness however, identical in its clinical characteristics and about as frequently as was produced by the injection of immune serum.

Since it is possible to produce in the rabbit, upon injection of a single large dose of normal or immune serum, a condition closely resembling or identical with serum sickness in man,[3] an attempt was made to compare the antigenicity (as measured by precipitin response and appearance of skin sensitivity) of normal horse serum with that of immune serum, as produced by a single subscapular injection in this species. Three animals were injected with pooled normal horse serum, 5 with the pseudoglobulin fraction of this normal horse serum, 3 with immune serum (collected from the same horses after immunization with diphtheria toxin and containing 1700 units of diphtheria antitoxin per cc.) and 5 with the pseudoglobulin fraction derived from this immune serum. Serum sickness occurred only in some of the animals receiving 75 mg. or more of serum nitrogen per kilo of body weight, and with about the same incidence and severity in those receiving normal serum as in those receiving immune serum. Skin sensitivity and precipitin production resulting from these injections are given in Table I. Skin tests were made by intradermal injection of 0.1 cc. amounts of various dilutions of normal and immune sera or the pseudoglobulin fractions of these sera in the cases of those animals receiving a single large dose of pseudoglobulin fraction. Tests were made for precipitin content of rabbit serum against

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[1] Tuft, L., and Ramsdell, S. G., *J. Immunol.*, 1929, **16**, 411.

[2] Tuft, L., and Ramsdell, S. G., *J. Exp. Med.*, 1929, **50**, 431.

[3] Fleisher, Moyer S., and Jones, Lloyd, *J. Immunol.*, 1933, **24**, 369.

TABLE I.

Rabbit No.	Horse Serum	Mg. serum N. inj. per kilo. body wt.	Highest dilution of serum or serum fraction giving positive skin reaction in 0.1 cc. amount		Days after inj.	Precipitin titre of rabbit serum for horse serum or fractions		Days after inj.
			Normal	Immune		Normal	Immune	
3	Whole Normal	100	100	100	30	10,240	10,240	9
4	Whole Immune	100	100	100	30	5,120	10,240	9
5	Whole Normal	100	1000	1000	24	10,240	10,240	12
6	Whole Immune	100	1000	1000	24	10,240	10,240	12
1	Whole Normal	50	100	100	30	10,240	10,240	12
2	Whole Immune	50	100	100	30	20,480	20,480	12
7	Pseudoglob. Normal	100	100	100	13	20,480	20,480	10
8	Pseudoglob. Immune	100	100	100	13	20,480	20,480	10
9	Pseudoglob. Normal	75	100	100	14	10,240	10,240	10
10	Pseudoglob. Immune	75	100	100	14	10,240	10,240	10
11	Pseudoglob. Normal	75	100	100	17	20,480	20,480	11
12	Pseudoglob. Immune	75	100	500	17	10,240	10,240	11
17	Pseudoglob. Normal	5	100	100	20	10,240	10,240	11
14	Pseudoglob. Immune	5	100	100	17	10,240	10,240	9
15	Pseudoglob. Normal	5	100	100	20	10,240	10,240	11
16	Pseudoglob. Immune	5	100	100	20	10,240	20,480	11

normal and immune horse sera, or their pseudoglobulin fractions in those cases where the animals received a single large dose of pseudoglobulin.

The results indicate that under these conditions skin sensitivity and precipitin production occur in the rabbit and to practically the same extent whether normal or immune serum is used as the antigen

7054 P

Intranuclear Inclusions in Salivary Glands of the Mole.*

L. E. AND ELEANOR J. RECTOR. (Introduced by E. V. Cowdry.)

From the Department of Anatomy, Washington University School of Medicine.

Intranuclear inclusions like those caused by herpes and other viruses are frequently discovered when tissues are carefully examined. Evidence that their production is the result of the action of any particular virus is often lacking. It has seemed desirable, therefore, to examine a large series of animals to determine the approximate distribution of such inclusions. We have found intranuclear inclusions to be constantly present in the salivary glands of 12 moles collected near St. Louis. These have been compared with the intranuclear inclusions in the salivary glands of guinea pigs and of humans, and with others recently reported in the same location in rats by Thompson. This comparison has been facilitated by the use of preparations of the human salivary glands kindly sent to Dr. E. V. Cowdry by Dr. S. B. Wolbach, and of rats by Dr. Juanita Thompson. The intranuclear inclusions in the moles resemble them in several particulars. The cells involved are greatly hypertrophied, so much so that they may easily be identified at comparatively low magnifications. The intranuclear inclusions themselves are conspicuous objects which stain with basic as well as with acid dyes and are separated from the surrounding nuclear membranes by distinct halos. There was but slight evidence of pathologic change in the tissue near the inclusions, and the moles appeared to be normal, exhibiting no symptoms attributable to virus action. Many attempts were made, by injecting the salivary glands of the moles into laboratory animals, to isolate a virus which might be responsible for the production of inclusions, but without success.

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* Aided by a grant from the Rockefeller Foundation for research in virus diseases.

7055 P

A Late Period of Subnormal Irritability Following Nerve Response.

HELEN TREDWAY GRAHAM.

From the Department of Pharmacology, Washington University School of Medicine.

The described effects of the passage of an impulse through isolated frog nerve on the irritability of the nerve are (1) the appearance of refractoriness immediately following the response and (2) under certain circumstances the appearance of supernormality, succeeding the refractoriness, and of much longer duration. By means of certain pharmacological agents, a third, still later effect may be made to appear, a depression of irritability differing from refractoriness not only in its time relations but in other respects as well. Yohimbine is the most effective agent as yet found for producing this effect, which will be referred to as "late subnormality".

About an hour after 1/20,000 yohimbine hydrochloride has been applied to the isolated sciatic nerve of *Rana pipiens*, cathode ray oscillograph observations very clearly reveal the 3 successive phases of irritability modification—refractory period, supernormal period, late subnormal period. The relatively refractory period is somewhat (0.5-1.0σ) longer than in the untreated nerve displaying supernormality; the supernormal period thus begins later than in the untreated nerve, while it ends considerably earlier. The maximum degree of supernormality is reduced. In one typical case, the maximum supernormality fell from 104.5% to 103%, and the duration of the supernormal period decreased from 138σ to 47σ. The supernormal period is immediately succeeded by the late subnormal period which lasts 5-10 seconds after the conditioning shock in the nerve yohimbinized as described. The minimum irritability during this period occurs 1-2 seconds after the shock, and after a single conditioning shock is usually 97-99% of the resting level in these lightly poisoned nerves. The subnormality may be both prolonged and intensified by stronger yohimbinization. When the drug action is strong enough to reduce the irritability during the subnormal period to about 95% of the resting level, the supernormality has ordinarily been largely or entirely removed, but there remains in the curve of recovery of irritability a crest of irritability in the region of former supernormality, and this crest tends to persist in less marked form even in deeply poisoned nerves. In these nerves the late subnormality has been observed to last 16-32 seconds.

A series of shocks, each one timed to fall in the late subnormal period of its predecessor, increases and prolongs the subnormality very markedly. Each successive shock of the series adds something to the depression left by preceding shocks, but each successive shock adds less than its predecessor. The level of minimum irritability eventually reached has been observed to be 50-96% of the resting irritability, varying with the rate of stimulation as well as with the degree of yohimbinization.

This increase of subnormality with repeated stimulation distinguishes the late subnormal period from the refractory period in which the recovery of irritability is described as following the same curve after one shock or after a series of shocks each timed to fall in the relatively refractory period of its predecessor. Late subnormality also differs from refractoriness in that the ability of the nerve to respond is not decreased; the height of maximum response is undiminished during the late subnormal period, even when the depression of irritability after one conditioning shock is considerable, or when it is enormously increased by a series of shocks. Late subnormality thus resembles supernormality in being a phenomenon manifested in change of irritability but not in change of ability to respond.

Yohimbine produces certain other changes in nerve function. The absolutely refractory period is usually lengthened, particularly with the higher concentrations of yohimbine; and the height of response tends to fall. The resting irritability is not significantly affected until the poisoning is very marked, when it may fall considerably. In keeping with all earlier experience linking supernormality and after-potential, yohimbine decreases and eventually removes the after-potential.

Of the other substances whose action has been examined for the production of late subnormality, cocaine (1/20,000-1/5000), procaine (1/7500-1/5000) and quinine (1/5000-1/1000), all applied as the hydrochlorides, produce conditions similar to the one described for yohimbine, except that the subnormality is less in degree and does not last so long, usually ending in about half a second. The minimum falls correspondingly earlier than the yohimbine minimum. Further, these drugs tend to produce relatively greater removal of supernormality so that the irritability changes are less striking. The effect of repeated shocks on subnormality has not been investigated on nerves treated with these drugs.

7056 P

Microincineration of Degenerating Anterior Horn Cells in Experimental Poliomyelitis.*

W. E. PATTON. (Introduced by G. H. Scott.)

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The literature concerned with poliomyelitis seems to be largely composed of results of studies on the more general and gross pathological aspects of the disease. Another line of investigation has been opened up by Marinesco, Policard and Cowdry which begins with the cell rather than the symptom. As yet, no such approach has been made toward the understanding of poliomyelitis. The present paper deals with but a single phase of the cytopathology of the disease—the changes in inorganic constitution as determined by the microincineration method.

The material was obtained from autopsies of 43 monkeys *(Macacus rhesus)* experimentally infected with poliomyelitis by the intracerebral and intranasal routes. The tissues were fixed in absolute alcohol formalin and prepared for incineration according to the method described by Scott.[1] Alternate serial sections were incinerated or were stained with hematoxylin and eosin in such a manner that colored and ashed preparations of the same cell were available for comparison. For controls, similarly prepared material from 10 normal monkeys or monkeys dying of diseases other than poliomyelitis was used.

The normal anterior horn cell incinerated under optimum conditions leaves a characteristic and uniform ash. The cytoplasm is represented by a very finely divided, mist-like, bluish or whitish ash. Against this filmy background spots of somewhat denser whitish, powdery material may usually be seen, evidently representing the Nissl bodies. The nucleus appears as a hole in the cell, set here and there with refractile granules, sometimes yellowish, but more often white, which may be identified as the chromatin substance. The mineral residue of the nucleolus is seen as a flat white, finely granular material of varying intensity.

In the spinal cord of a monkey inoculated with poliomyelitis virus,

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* Aided by (1) a grant from the Milbank Fund for the study of Infantile Paralysis and (2) a grant from the Rockefeller Foundation for research in virus diseases.

[1] Scott, G. H., *Protoplasma*, 1933, **20**, 133.

several forms of degenerating cells may be seen. Three typical pictures will be described here:

1. During the early stages of degeneration the cell becomes somewhat swollen, the Nissl bodies are lost and the cytoplasm tends to take a lighter stain. The nucleus is often enlarged and may occupy an excentric position in the cell body. The basophilic chromatin material appears bead-like or as irregular masses scattered about within the nucleus or plastered to the nuclear membrane. It is in such cells that the nuclear inclusions described by Covell[2] and Hurst[3] may be seen. Incinerations of these cells show that they contain slightly less mineral matter than the normal neurone. The Nissl substance does not appear. Although the inclusion bodies are too small to be identified in consecutive sections, they seem to leave a very slight ash.

2. In more severely injured cells the cytoplasm becomes filled with basophilic granules. These are scattered about within the cell or may be localized in one portion. They are globular in shape and vary in staining properties from a deep blue to a dense black. The nucleus is homogeneous, hyperchromatic and shrunken. The incinerated preparation of such a cell is indeed striking. It is characterized by a dense white, refractile, granular cytoplasmic ash which seems to be piled on the slide. This enormous increase in mineral matter is shared by the processes, which appear as glistening streaks in the gray matter of the cord. The nucleus, in spite of its marked increase in stainable substance leaves a very light ash, in which a mass of mineral matter resembling that left by the nucleolus may sometimes be seen.

3. The cytoplasm of the completely necrotic cell appears as a spongy acidophilic material, usually containing a round, acidophilic, hyaline body which represents the nucleus. Such a cell when incinerated leaves a net-like or granular ash which is somewhat heavier than that left by a normal cell. The hyaline nucleus leaves no mineral residue whatever.

Neuronophagia may take place during either of the 2 last described stages. In either case the cytoplasm of the neuronophages takes on the staining properties of the phagocytized cell, *i. e.,* it may contain basophilic granules or may exhibit the acidophilic staining properties of the necrotic cell. The ash of the neuronophagic focus resembles that of the degenerating cell except that it is dotted with the nuclei of the phagocytes. The granular cells, after incineration leave the characteristic heavy refractile deposit, while the acidophilic cells are represented by a white net-like ash. It would seem that little or no change in inorganic material takes place during neuronophagia, the entire mineral content of the degenerating cell being taken over by the phagocytes.

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[2] Covell, W. P., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 927.

[3] Hurst, E. W., *J. Path. and Bact.,* 1929, **32**, 457.

Western New York Section.

Clifton Springs Sanitarium, October 21, 1933.

7057 P

Effect of Atebrine on Avian Malarias.*

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Atebrine is a new anti-malarial drug recently developed in Germany, first tested on avian malaria, and the results reported by Kikuth.[1] He used *Plasmodium praecox, elongatum* and *circumflexum,* and *Haemoproteus orizivorae* (a parasite of the rice-finch), and found that it was effective in all cases, but that it would not sterilize malarial infections. Used with plasmochin, however, it either prevented relapses entirely or very much delayed them in the case of *Haemoproteus.* Whether any of the latter cases were sterilized is not stated. He concluded that atebrine is particularly active against the schizonts, in contrast to plasmochin which works best against the gametocytes.

The work herein reported deals only with the effect of atebrine on *Plasmodium rouxi, circumflexum,* and *cathemerium.* The purpose has been to discover whether clearly marked specific differences, such as those already demonstrated by the author[2, 3, 4] for plasmochin and quinine in the case of these 3 species and 2 others *(elongatum* and *praecox),* could be demonstrated for this drug also, and to determine as accurately as possible how the latter compared in effectiveness with quinine and plasmochin.

The drug has been administered orally in all cases, and the period of treatment has varied from 7 to 9 days (as compared with 2 weeks, or sometimes longer, in the experiments with the other drugs cited above). The treatment period was shortened because the drug ap-

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* The author desires to acknowledge his indebtedness to the National Research Council for financial assistance.

[1] Kikuth, Walter, *Deutsche Med. Wochensch.,* 1932, **14**, 1.

[2] Manwell, R. D., *Am. J. Trop. Med.,* 1930, **10**, 379.

[3] Manwell, R. D., *Am. J. Trop. Med.,* 1932, **12**, 123.

[4] Manwell, R. D., *Am. J. Trop. Med.,* in press.

peared to cause lesions about the mouth but the birds recovered promptly after administration of the drug was stopped. The dosage used was 2 mg., dissolved in 100 cm. of water, and this amount was given twice a day. The results were checked by periodic blood examinations extending over a period of 2 months (3 months in the case of *Plasmodium rouxi*), and by subinoculation of blood from the treated birds into clean birds, when it appeared that no relapses occurred during the period of observation. Female canaries were used in all cases. Some birds were treated during the incubation period, others during the acute stage, and (in the case of *Plasmodium rouxi*) some were given repeated doses of parasites during the period of treatment in order to determine the value of the drug as a prophylactic against this type of infection. All infections were produced by blood inoculation.

The results can be briefly summarized. It was not possible in any case to prevent or cure infections with *Plasmodium circumflexum* and *cathemerium,* but atebrine appears to be a perfect specific against infection with *Plasmodium rouxi,* at least when parasites are introduced by blood inoculation. No bird (of the 16 treated) developed any infection afterward. It is recognized that the series was relatively small and work is being carried on, both with the other species and additional cases of *rouxi,* but the results are so consistent that it is believed that they are significant enough to report. So far the treatment of mosquito-induced infections has not been tried, and it is quite possible that the results of this sort of therapy would be different, in view of the experiments recently reported by Russell and Nono[5] and Tate and Vincent.[6] Including controls, 50 birds have been used in the work on which this paper is based. Twenty-one were used in the case of *Plasmodium rouxi,* 19 of *cathemerium* and 10 of *circumflexum.*

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[5] Russell, Paul F., and Nono, Andres M., *Phil. J. Sci.*, 1932, **49**, 595.

[6] Tate, P., and Vincent, M., *Parasitology*, 1933, **25**, 96.

7058 P

Experimental Hypertrophy of the Adrenal Glands.

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The relationship between the hypophysis and the adrenal glands which has long been known to exist in clinical cases,[1] has not been well understood. Experiments in animals have thrown considerable light on this subject especially in regard to a pituitary substance necessary for normal adrenal function.[2] Many studies on pituitary implants and extracts in relation to the reproductive system have been made in recent years but the effects on the adrenal glands, when reported, were not significant. In immature rats implanted with pituitary glands of castrated rats some hypertrophy of the adrenals was noted at autopsy but the results were not outstanding.[3] Recently we have continued these studies and have arranged the experiments to show the effects of the cortico-adreno-tropic substance of the pituitary. The results have been very striking and consistent.

Albino rats weighing about 150 gm. were divided into 2 groups of equal weight; one group consisted of 35 controls which were not injected; the other group consisted of 35 rats which were injected daily for 10 days with an extract of the pituitary gland. On the eleventh day the rats were killed and the adrenals weighed. The hypertrophy of the adrenal glands produced in the injected group amounted to as much as 150% and as little as 20% in the individual rats. Accurate determinations show that this hypertrophy is largely confined to the cortex which consists of cells larger than normal and more filled with lipoid material. Experiments with the growth hormone of the pituitary showed some enlargement of the adrenals but the effects were slight in comparison with whole pituitary extract.

The thyroid gland was not increased in size.

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[1] Calder, R. M., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 87.

[2] Evans, H. M., *J. Am. Med. Assn.*, 1933, **101**, 425.

[3] Emery, F. E., *Endocrin.*, 1933, **17**, 64.

7059 C

Water Shift in the Muscle of Adrenalectomized Rats.*

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From the Department of Physiology, University of Buffalo.

Animals with adrenal insufficiency suffer disturbances in ability to regulate the water and electrolyte content of their tissues and plasma. Increase has been reported in the water content of muscle, liver, and skin,[1, 2, 3] and decrease in the plasma volume.[4, 5, 6, 7] The adrenalectomized animal cannot shift water as readily under stresses as does the normal animal.[1] The excretion of water is less, and of chlorides more[2]; there is a change in the electrolyte content of the plasma,[8] and a disturbance in electrolyte balance.[9]

Viale and Bruno[5] suggested that the changes in water content of tissues and plasma might be due to an increase in permeability. The present study was undertaken to determine whether any evidence of a change in permeability could be found in the tissues of animals suffering from adrenal insufficiency. The first series of experiments have dealt with the swelling and shrinking of tissues from normal and from adrenalectomized rats in balanced solutions of varying strengths. According to Gellhorn,[10] a close parallelism frequently exists between imbibition and permeability.

Muscles of normal and adrenalectomized albino rats weighing about 150 gm. were employed in this study. Adrenalectomized animals were used 4 to 7 days after the operation; only those animals giving evidence (loss of weight) of adrenal insufficiency were used.

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* Aided by a grant from the Carnegie Corporation of New York to the Carnegie Institution of Washington.

[1] Hartman, F. A., Brownell, K. A., and Lockwood, J. E., *Endocrinology*, 1932, **16**, 521.

[2] Silvette, H., and Britton, S. W., *Am. J. Physiol.*, 1933, **104**, 399.

[3] Hartman, F. A., *Annals Int. Med.*, 1933, **7**, 6.

[4] Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1926, **78**, 711.

[5] Viale, G., and Bruno, A., *Compt. Rend. Soc. de Biol.*, 1927, **97**, 261.

[6] Rowntree, L. G., and Snell, A. M., A Clinical Study of Addison's Disease, Philadelphia, 1931.

[7] Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., and Parkins, W. M., *Science*, 1933, **77**, 58.

[8] Loeb, R. F., *Science*, 1932, **76**, 420.

[9] Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

[10] Gellhorn, E., 1929, *Das Permeabilitätsproblem*, Berlin, (cited in Gellhorn, E., *Am. J. Physiol.*, 1931, **96**, 203.

Animals obviously moribund were discarded. Under ether anesthesia, the m. extensor digitorum longus was dissected out, quickly weighed in a glass-stoppered weighing vial, then placed in aerated balanced salt solution at a temperature of 37.5° C., and with a *pH* of about 7.3. At definite time intervals, the muscle was lifted out, quickly dried on filter paper, weighed, and returned to the solution.

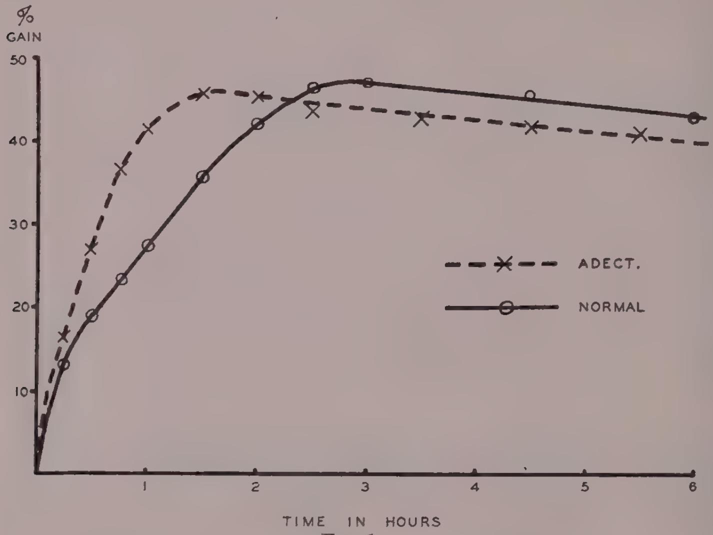

FIG. 1.

Changes in weight of muscles in hypotonic solution.

Fig. 1 shows the results obtained by such a procedure in a hypotonic solution (73 parts Locke's solution* to 27 parts distilled water). Muscles from adrenalectomized animals imbibe water considerably faster than those from normal animals; the maximum water uptake is about the same in either case, but is reached much sooner in the case of muscles from the adrenalectomized rats.

Fig. 2 shows the change in weight of muscles in a strongly hypertonic solution (balanced Locke's solution, but with 3 times the usual salt concentration). The figure shows that the muscles from adrenalectomized animals lose weight faster in the beginning than do those from normal animals, that they reach the point of minimum weight more quickly, and that once they start to gain weight, they gain more rapidly.

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* Locke's solution of the following composition was used: NaCl, 0.9; KCl, 0.042; $CaCl_2 \cdot H_2O$, 0.024; $NaHCO_3$, 0.02; H_2O, to 100. Buffer used: NaH_2PO_4 plus NaOH.

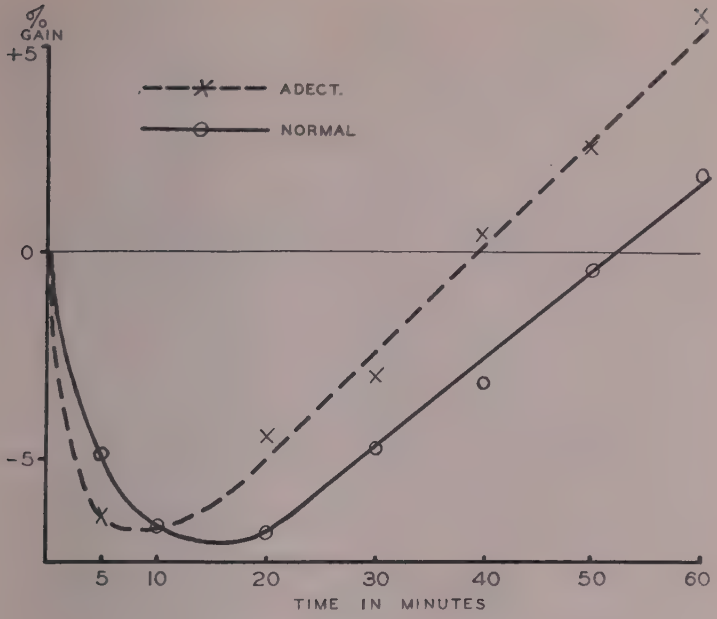

FIG. 2.
Changes in weight of muscles in hypertonic solution.

In all cases, the points on the curves represent averages of 10 or more determinations. Altogether, 51 muscles from 26 normal animals and 40 from 20 adrenalectomized animals were used in this study.

Comparable results were obtained in solutions intermediate in strength; at a salt concentration 30% greater than normal Locke's solution, normal muscles neither gain nor lose appreciably in the first few minutes; muscles from adrenalectomized animals begin to gain immediately. A difference in the rate of change of weight was found in all concentrations tried, with the exception of distilled water. Probably the muscles are injured so rapidly in distilled water that any differences existing in the 2 sets of muscles are obscured.

Changes in weight of a muscle in balanced salt solution represent mostly a shift of water into or out of the muscle; it is evident that this shift occurs more readily in either direction in a muscle from an adrenalectomized animal than in a normal one. It is reasonable to suppose that this change may be due to an increase in permeability in the former.

7060 C

Parallel Adsorption of Crystalline Pepsin and Peptic Activity Upon Casein and Ovalbumin.*

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Waldschmidt-Leitz and Kofrányi[1] found that by repeatedly shaking a solution of crystalline pepsin with small quantities of suspension of cantaloupe seed globulin they could adsorb the peptic activity while the crystalline pepsin-protein was left in solution. They accordingly claim that Northrop's[2] crystalline pepsin is simply a case of an enzyme adsorbed upon a protein. Their evidence for the non-adsorption of the pepsin-protein was obtained by determining the weight of dry matter left after centrifuging off the suspended material and by blank determinations made with cantaloupe globulin alone.

It is unfortunate that these authors did not employ a specific test for the protein of crystalline pepsin, for their centrifuged solutions may have contained not only crystalline pepsin, but also cantaloupe globulin in quantity not indicated by blank determinations, as well as digestion products. By the employment of a test which is specific for crystalline pepsin-protein, provided other proteins are not present in the solution, I have been able to demonstrate that when the peptic activity of a solution of crystalline pepsin is completely adsorbed upon casein or coagulated ovalbumin the pepsin-protein is also completely adsorbed. Furthermore, I have found that the rates of adsorption of peptic activity and of pepsin-protein are the same. These findings conclusively disprove the work of Waldschmidt-Leitz and Kofrányi unless one is willing to make the highly improbable assumption that crystallized cantaloupe seed globulin possesses some peculiar action upon pepsin which is not exerted by casein or by denatured ovalbumin.

Casein and coagulated ovalbumin were chosen as adsorbents because of their insolubility at pH 4.8. Edestin could not be used since it was too soluble below pH 5 and it was not desirable to subject pepsin to solutions less acid than this. Coagulated edestin

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* This work was aided by a grant from the Sage Research Fund of Cornell University.

[1] Waldschmidt-Leitz, E., and Kofrányi, E., *Naturwiss.*, 1933, **10**, 206.

[2] Northrop, J. H., *J. Gen. Physiol.*, 1930, **13**, 739.

formed large lumps which could not be pipetted in suspension. The casein was prepared by washing 10 gm. of Merck's casein repeatedly with dilute acetate buffer of pH 4.8. The casein was then mixed with 90 cc. of water and 10 cc. of 0.5 M acetate buffer of pH 4.8. The ovalbumin was prepared by heating 1000 mg. of recrystallized,

EXP. I.
Adsorption of pepsin and pepsin-protein on casein.

Solution	cc. used for adsorption	$[\mathrm{PU}]^{\mathrm{Hb}}$	$[\mathrm{PU}]^{\mathrm{Hb}}$ as %	pepsin-protein as %
Original	100	.0115	100	100
Filtrate I	91	.0077	67	60
" II	75	.0045	39	40
" III	72	.0021	18	20
" IV		.00085	7.3	10

EXP. II
Adsorption of pepsin and pepsin-protein on ovalbumin

Solution	cc. used for adsorption	$[\mathrm{PU}]^{\mathrm{Hb}}$	$[\mathrm{PU}]^{\mathrm{Hb}}$ as %	pepsin-protein as %
Original	100	.0110	100	100
Filtrate I	91			
" II	77	.0063	57	50
" III	72	.0038	34	30
" IV		.0018	16	20

EXP. III.
Adsorption of pepsin and pepsin-protein on ovalbumin.

Solution	cc. used for adsorption	$[\mathrm{PU}]^{\mathrm{Hb}}$	$[\mathrm{PU}]^{\mathrm{Hb}}$ as %	pepsin-protein as %
Original	100	.0096	100	100
Filtrate I	95	.0066	69	70
" II	89	.0042	44	40
" III	82	.0020	21	20
" IV		.00012	1.2	0.8

dialyzed ovalbumin in 90 cc. of water for 15 minutes, cooling and adding 10 cc. of acetate buffer. Both proteins were suspended by shaking just before pipetting. The casein was lumpy and difficult to pipette.

Crystalline pepsin solutions were made by dissolving pepsin crystals in a little water containing 10 cc. of acetate buffer, diluting to 200 cc. and filtering. The pepsin employed in Exp. I and II was once crystallized; the pepsin ued in Exp. III was twice crystallized. The pepsin concentrations (N x 6.54) were respectively 45, 48, and 37 mg. per 100 cc. in Exp. I, II, and III.

Adsorption was carried out by adding 5 cc. of suspended protein to 100 cc. of pepsin at room temperature. After rotating for

6 minutes the material was filtered and the filtrate tested for peptic activity and for pepsin-protein; the remaining filtrate was then used for a second adsorption. For determination of peptic activity the method of Anson and Mirsky[8] was employed and the values are expressed as their pepsin units, or $[PU]^{Hb}$. When solutions of low peptic activity were tested it was necessary to digest for intervals longer than the usual 5 minutes.

Pepsin-protein was estimated by adding one drop of 10% sodium hydroxide to 5 cc. of the clear pepsin solution, followed, after mixing, by 1 cc. of 0.5 N sulfuric acid. The suspension of denatured pepsin-protein was then compared with various dilutions of the original pepsin solution which had been coagulated in the same manner. With this test it was found possible to make approximate estimation of as little as one part of crystalline pepsin-protein in 300,000 of water, using a beam of light in a dark room. The results of 3 experiments are shown in the tabulation.

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[8] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.

New York Meeting.

New York Academy of Medicine, November 15, 1933.

7061 P

Endogenous Glycine Formation in Myopathies and Graves' Disease.

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The relation between glycine and creatin metabolism, first pointed out by Brand[1] and his associates, and extended by the therapeutic investigations of Milhorat, Thomas, and Techner,[2, 3] makes the question of the endogenous formation of glycine in disorders of creatin metabolism of considerable significance. In addition to the well recognized myopathies such as progressive muscular dystrophy and myasthenia gravis, abnormal excretion of creatin occurs in other diseases associated with muscular weakness, notably Graves' disease.

In a recent study[4] of the character of the creatin disturbance in progressive muscular dystrophy and Graves' disease, we found a rather striking similarity between the two disorders. In both there was (1) a diminished creatin tolerance, (2) an augmentation of the creatinuria following the ingestion of glycine, and (3) a similarity in the lesions produced in the skeletal musculature (from the pathological studies of Askanazy,[5] Naffziger,[6] and others).

We also studied[4] the rate of endogenous glycine formation as measured by hippuric acid excretion following the ingestion of sodium benzoate (method of Quick[7]), and found it to be unimpaired in both these diseases. Using the same method of investigation Freidberg and West[8] recently found that children with pseudohypertrophic muscular dystrophy were also able to form hippuric acid at the normal rate.

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[1] Brand, *Am. J. Physiol.*, 1929, **90**, 296.

[2] Milhorat, A. T., Thomas, K., and Techner, F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 607.

[3] Milhorat, A. T., Thomas, K., and Techner, F., *Deutsch. Arch. f. Klin. Med.*, 1933, **174**, 487.

[4] Proc. 25th annual meeting of Am. Soc. for Clin. Invest., May 8, 1933. *J. Clin. Invest.*, 1933, **12**, 966.

[5] Askanazy, *Deutsch. Arch. f. Klin. Med.*, 1898, **61**, 118.

[6] Naffziger, *Arch. Ophthalm.*, 1933, **9**, 1.

[7] Quick, *J. Biol. Chem.*, 1931, **92**, 65.

In the following paragraph are assembled the data from which our conclusions were drawn. They include normal controls and later studies of other myopathies. In addition to determining hippuric acid output, the excretion of glucuronic acid was also followed, since this substance might have been available for conjugation with benzoic acid in the case of a defect in glycine formation. Daily creatin and creatinine determinations were made with the subjects on creatin-creatinine-free diets in order to observe the effect of the stimulation of glycine formation and its subsequent diversion.

The hippuric acid excretion during the 4 hour period after the ingestion of 5.9 gm. of sodium benzoate, ranged from 4.3 to 6.4 gm. in 10 normal controls. Six patients, with Graves' disease and creatinuria, excreted between 3.7 and 6.0 gm. of hippuric acid during a similar period, with no significant differences after the abolition of the creatinuria by iodine. Repeated tests on 4 adults with progressive muscular dystrophy gave the following results: Case 1 (mild) 5.1, 5.6, 5.7 gm.; Case 2 (advanced) 4.2, 4.9, 4.3 gm.; Case 3 (advanced) 5.5, 5.5, 5.6 gm.; Case 4 (advanced) 4.4 gm. A miscellaneous group of myopathies gave these results: myotonia congenita, 5.8 gm.; myotonia atrophica, 4.9 gm.; periodic family paralysis (age 14) 3.7 gm. The only abnormally low result was obtained in a case of myasthenia gravis in which the excretion was 2.6 and 3.1 gm. on 2 tests.

Conclusions. Endogenous glycine formation following benzoate ingestion fell within normal limits in progressive muscular dystrophy, Graves' disease, and the other myopathies studied; except for one case of myasthenia gravis in which it was below normal, but possibly not significantly so. This does not exclude the possibility that the normal stimulus to glycine formation is inadequate in these diseases. There was no unusual excretion of glucuronic acid.

In several instances of progressive muscular dystrophy and Graves' disease, significant increases (70 to 310 mg.) in the excretion of creatin occurred on the day on which the benzoate was administered. This may have been due to the presence of increased amounts of glycine in the blood stream preliminary to its conjugation with benzoic acid, comparable to the effect of the ingestion of glycine. Or, what is more likely, it may have resulted from the loss of glycine through the urine, with an exacerbation of the defect in the creatin metabolism.

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[8] Freidberg and West, *J. Biol. Chem.*, 1933, **101**, 449.

The normal synthesis of glycine in these patients and the ready formation of creatin when they ingest glycine, tend to place the defect in creatin metabolism beyond these stages. The inference is that the disturbance is located in the muscle itself, probably in the enzyme system which controls the breakdown and building up of phosphocreatin. The thyroid hormone is apparently involved in this process.

7062 P

The "Q" Deflection in Normal and Abnormal Human Electrocardiograms.

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(Introduced by Harry Gold.)

From the Division of Cardiology, ''B'' Medical Services, the Jewish Hospital of Brooklyn.

Recent literature[1, 2, 3, 4] contains much work on the significance of the Q-wave, and, more particularly of a large Q-3. Attempts have been made in some of these studies[1, 4] to explain the mechanism underlying the production of a large Q-wave. Our knowledge of the mechanism of the normal Q-wave (*e. g.*, Lewis' and Wilson's theories) has been used as a basis for discussions of the abnormal Q-waves. Conclusions have been drawn by one author[4] from pathological correlations that the large Q-wave is representative of changes in a well localized portion of the heart (the left half of the septum posteriorly). Insufficient emphasis has been placed on the fact that the names Q, R and S are entirely arbitrary and that the Q-R-S system of terminology frequently gives the same name to parts of the electrocardiogram in the 3 standard leads that do not correspond in time, and different names to parts that do so correspond. We attempted an analysis of the Q-wave in normal records and in records of the large Q-3 type (described by Pardee[1]) to determine the relative time occupied by this deflection in the belief that this time data would throw light on the significance of the Q-wave in normal and abnormal electrocardiograms. We obtained our time data by using Einthoven's formula: *i. e.,* when on any and every vertical line the electrocardiographic curves can be superimposed so that the height in Lead I added algebraically to that in Lead III gives the height in Lead II, the time relations are identical.

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[1] Pardee, H. E. B., *Arch. Int. Med.*, 1930, **46**, 470.

[2] Willius, F. A., *Am. Heart J.*, 1931, **6**, 723.

[3] Carr, F. B., Hamilton, B. E., and Palmer, R. S., *Am. Heart J.*, 1933, **8**, 519.

[4] Fenichel, N. M., and Kugell, V. H., *Am. Heart J.*, 1931, **7**, 235.

Results. In each of 25 normal ventriculograms Q-1, Q-2 and Q-3 corresponded in time with reasonable accuracy, suggesting that the Q-wave represented a more or less fixed physiological phenomenon in these instances. In ventriculograms of the large Q-3 type (fulfilling Pardee's criteria[1]) Q-3 did *not* correspond in time with Q-1 or Q-2. The time represented by Q-3 in 50 of these tracings (taken from 1200 consecutive electrocardiograms) was in *no* case identical with that occupied by Q-1 and Q-2. In *most* of these tracings Q-3 corresponded in time with parts of R-1 and R-2. In many of these tracings Q-3 corresponded more or less accurately in time with R-1 or R-2.

The results given above indicate that the *large* Q-3 is not comparable with the normal Q-wave in that it represents a grossly varying time interval after the beginning of the Q-R-S and cannot, therefore, represent a fixed phenomenon in the heart.

In studying the electrocardiograms of the large Q-3 type it was apparent that the "R" and "S", and possibly "P", as well as "Q" waves, were altered in a characteristic way. As Q-3 got larger (*e. g.,* with respiratory changes) R-3 became smaller, S-3 disappeared and P-3 occasionally became inverted. These facts suggested some alteration of the electrical axis of the ventricle as a whole rather than of some limited portion of it, and at times of the electrical axis of the auricle as well. Meek and Wilson[5] showed that rotation of the dog's heart about a longitudinal axis produced a large Q-wave. This work suggested that a large "Q" in humans might be indicative of a rotation of the ventricular electrical axis about a longitudinal axis; rotation to the left producing a large Q-3, and rotation to the right a large Q-1. Such a thesis appeared to explain all the known facts about the large Q-wave—including its relative frequency in cases with coronary artery disease,[1] in pregnant women,[3] in normal infants[6]; and its occasional occurrence in normal adults.[7]

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[5] Meek, W. J., and Wilson, A., *Arch. Int. Med.,* 1925, **36**, 614.

[6] Seham, M., *Am. J. Dis. Child.,* 1921, **21**, 247.

[7] Ziskin, Thos., *Arch. Int. Med.,* 1932, **50**, 435.

Rotation of the ventricular electrical axis could be the result: (a) of mechanical factors that change the position of the ventricle; (b) of a change in the position of the leads that causes a relative rotation of the heart; (c) of damage to one part of the ventricular musculature with collapse, the contraction of scar tissue, or dilatation of the damaged area; (d) of thickening of one part of the ventricular musculature.

The work of Pardee,[1] Willius[2] and others suggests that most large Q-waves are caused by the mechanism described in "C" above, but in any particular electrocardiogram there is no more reason for considering a large Q-3 or a large Q-1 of itself indicative of coronary artery or myocardial disease than there is for regarding a deep S-3 as always indicative of hypertrophy of the left ventricle.

7063 P

Antistreptolysin Titre of the Serum in Acute Glomerular Nephritis.

DAVID SEEGAL AND JOHN D. LYTTLE. (Introduced by A. R. Dochez.)

From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian and Babies' Hospitals, New York City.

Todd[1] has demonstrated that the serums of animals immunized with *S. hemolyticus* develop a significant amount of an antibody which inhibits the activity of the streptococcal hemolysin. The serums of animals immunized to a number of other bacteria (pneumococcus, hemolytic staphylococcus, non-hemolytic streptococcus, and *B. diphtheriae*) do not contain appreciable quantities of this antihemolysin.

In subsequent studies Todd[2] working in conjunction with Coburn and Pauli[3] showed that there was a consistent increase in the antistreptohemolysin titre (termed antistreptolysin by Todd) in human serums *after* hemolytic streptococcus infections. This antibody did not appear in significant amounts in the serums of patients convalescing from such other infections as lobar pneumonia, hemolytic staphylococcus osteomyelitis, joint tuberculosis and measles. The presence of high antistreptolysin titres in the serums of patients with rheumatic fever was additional immunological evidence leading to Coburn and Pauli's hypothesis that *S. hemolyticus* initiates the rheumatic process.

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[1] Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267.

[2] Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

[3] Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 609.

Todd found that at least 0.01 cc. of the serum of a normal individual without a recent hemolytic streptococcus infection was required to neutralize the hemolysin unit he utilized. He observed that as little as 0.0008 cc. of the serum of patients convalescing from hemolytic streptococcus disease contained the same neutralizing dose of antistreptolysin. Todd has described the antihemolysin titres of serums by recording the reciprocals of the fractions of the neutralizing doses of the serums as units. Thus a neutralizing dose (N.D.) of 0.01 cc. is equivalent to 100 units of antistreptolysin. He considers this value the upper limit of normal in the serums of healthy individuals without a preceding hemolytic streptococcus infection.

TABLE I.
Antistreptolysin Titre of Sera in Acute Glomerular Nephritis.

	N.D.* cc.	Units of antistreptolysin	Preceding infection	Associated Organism	Days between serum withdrawal and onset of preceding infection	Days between serum withdrawal and onset of acute nephritis
1	.001	1000	Pharyngitis	Hem. Strep.	38	24
2	.003	333	Mastoiditis	" "	58	43
3	.007	144	Peritons. Abscess	" "	8	6
4	.006	166	" "	No Hem. Strep.	24	13
5	.001	1000	Pharyngitis	" " "	63	57
6	.008	125	Peritons. Abscess	Hem. Strep.	26	14
7	.01	100	" "	No Hem. Strep.	17	8
8	.002	500	Pharyngitis	" " "	22	13
9	.003	333	"	Hem. Strep.	28	10
10	.003	333	Sinusitis	" "	22	12
11	.002	500	Pharyngitis Cervical adenitis	" "	14	14
12	.004	250	Mastoiditis	" "	18	20
13	.007	144	Otitis media	" "	46	27
14	.003	333	Pharyngitis	" "	30	15
15	.0008	1250	Otitis media Cervical adenitis	" "	24	21
16	.004	250	Pharyngitis Cervical adenitis	No Hem. Strep.	21	11
17	.005	200	Sinusitis	Hem. Strep.	31	23
18	.005	200	Peritons. Abscess Cervical adenitis	" "	41	29
19	.005	200	Pharyngitis	No Hem. Strep.	43	20
20	.01	100	"	Hem. Strep.	52	38
21	.006	166	None	No Hem. Strep. in throat culture		30
22	.007	144	Otitis media	Hem. Strep.	29	19

*N.D. Fraction of a cc. of the patient's serum required to neutralize 2.5 Minimal Hemolytic Doses (M.H.D.) of streptolysin.
100 units of antistreptolysin is equivalent to the N.D. expressed as 0.01 cc.

The accompanying table shows the degree of the antistreptolysin titre* of the serum 6-57 days after the onset of acute glomerular nephritis in 22 unselected cases. A throat or upper respiratory infection preceded the onset of acute nephritis in 21 of these patients. Fifteen of these infections were associated with the presence of the hemolytic streptococcus in the pharynx.

Twenty of the 22 serums from this group of cases contain a sufficiently high antistreptolysin titre to be indicative of a recent hemolytic streptococcus infection. The remaining 2 serums contain 100 units each of the antibody. These data offer strong additional evidence to the concept that acute glomerular nephritis in New York City is chiefly related to a preceding hemolytic streptococcus infection.

7064 P

Blood Cytology of Normal Rabbits as an Index of Their Reaction to Experimental Syphilis.

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From the Laboratories of the Rockefeller Institute for Medical Research.

The possibility that the blood cytology might furnish indices of resistance and susceptibility to disease has been considered in the studies on the blood of rabbits carried out in this laboratory. The procedure has been to make a series of blood counts on groups of young adult male rabbits over a period of weeks or months; the animals were then inoculated with some pathogenic agent, or were kept under observation until a spontaneous disease, such as snuffles, developed. Upon the basis of careful clinical and postmortem examinations, the severity of the disease in each rabbit was quantitatively estimated. An attempt was then made to ascertain whether any relationship existed between the conditions found in the blood before inoculation and the severity of the disease which developed. For these analyses, the mean preinoculation blood cell values for each animal were used. In the case of a transmissible malignant tumor, certain definite relationships were found, some of which have been reported.[1] The present communication is concerned with results obtained in experimental syphilis.[2]

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* All the streptolysin determinations were made through the kindness of Miss Ruth H. Pauli.

Observations were made on 85 rabbits, comprising 9 experimental groups. Each animal was inoculated in one testicle with the Nichols' strain of *Tr. pallidum.* In each instance, lesions of the inoculated testicle developed, and in the majority of cases, of the uninoculated testicle also; of the 85 animals 46 developed and 39 failed to develop clinically recognizable bone or skin lesions during the 3 months' observation period. Upon the basis of the occurrence of these generalized lesions, the rabbits were classified as 39 resistant and 46 susceptible animals.

The resistant animals had before inoculation significantly higher hemoglobin, lower lymphocytes both per cmm. and in per cent, lower total white blood cells and higher neutrophiles in per cent than the susceptible animals. There were no statistically significant differences in the preinoculation mean values between the susceptible and resistant rabbits for the following blood factors: Total number of red blood cells and blood platelets, neutrophiles per cmm., and both relative and absolute numbers of basophiles, eosinophiles, and monocytes. (R.B.C., $+108{,}000 \pm 105{,}000$; hb., $+7.3 \pm 2.05$, $t = 3.58$, $P = 0.01$; platelets, $-29{,}800 \pm 40{,}000$, $n = 40$; w.b.c., -980 ± 458, $t = 2.14$, $P = 0.035$; neut. per cmm., -124 ± 234; baso. per cmm., $+50 \pm 115$; eosino. per cmm., -34 ± 26; lymph. per cmm., -774 ± 152, $t = 5.09$, $P = 0.01-$; mono. per cmm., -76 ± 106; neut. %, $+4.6 \pm 1.5$, $t = 3.00$, $P = 0.01-$; baso %, $+1.0 \pm 1.0$; eosino. %, -0.16 ± 0.27; lymph. %, -5.9 ± 1.36, $t = 4.33$, $n = 83$, $P = 0.01-$; mono. %, -0.39 ± 0.78, $n = 83$).

From the standpoint of the variance, or the scatter about the mean, the resistant animals had significantly larger values than the susceptible animals with respect to total white blood cells, neutrophiles and monocytes per cmm., and basophiles both per cmm. and in per cent, while a significantly lower value obtained for red blood cells. (W.B.C., $z = +0.3738 \pm 0.1557$, $n = 83$, $P = 0.02$; baso. %, $z = +0.4278 \pm 0.1557$, $n = 83$, $P = 0.01-$; neut. per cmm., $z = +0.3381 \pm 0.1557$, $n = 83$, $P = 0.03$; baso. per cmm., $z = 0.4673 \pm 0.1557$, $n = 83$, $P = 0.01$; mono. per cmm., $z = +0.3628 \pm 0.1557$, $n = 83$, $P = 0.02$; r.b.c. per cmm., $z = -0.3876 \pm 0.1557$, $n = 83$, $P = 0.015$). No differences between the resistant and susceptible animals were noted as to the variance for hemoglobin, blood platelets, eosinophiles and lymphocytes both per cmm. and in per cent, and for neutrophiles and monocytes in per cent ($P = 0.05+$).

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[1] Casey, A. E., and Pearce, L., *J. Exp. Med.*, 1931, **54**, 475.

[2] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 670.

When the relation of each blood factor to the severity of disease was plotted on a correlation table, the resulting relationships were strikingly non-linear, consisting of symmetrical and asymmetrical S, M, and U shaped curves. The red blood cells, the hemoglobin, the platelets, the total white blood cells, the neutrophiles, the basophiles, the lymphocytes, and possibly also the monocytes and the eosinophiles were all related in various degrees with the reaction of the host to experimental syphilis. In addition, no 2 blood elements had exactly the same relationship. These results indicate that the simple analysis by difference of the means and difference of the variance was not the most appropriate method of recording the degrees of relationship. The significant values obtained are, therefore, all the more remarkable in that the non-linearity was not taken into account.

It should be pointed out that all blood values observed in these experiments fall within the limits for normal rabbits. All animals appeared to be free from intercurrent disease and this feature determined their selection for these experiments. It would appear, therefore, that the hemocytological constitution as measured by preinoculation values has a statistically significant relation to the reaction of the rabbit host to experimental syphilitic infection.

7065 P

Strain Differences in Susceptibility to Tar-Induced Skin Tumors in Mice.

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We have already shown that certain strains of mice are more susceptible than others to tumors of the lung, both spontaneous and tar-induced. There is evidence also that these differences in susceptibility segregate out in appropriate crosses as would be expected if they were dependent upon Mendelian units. The present report concerns tar-induced tumors of the skin in mice.

The technique was the one usually employed in such experiments.[1]

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[1] *J. Exp. Med.*, 1925, **17**, 829.

A coal tar residue was applied to the skin of the mouse in the interscapular region 3 times per week for 4 months. After a latent period papillomas may arise which sometimes develop into carcinoma or malignant growths may develop without a marked papillomatous stage. The course of the experiment was followed by external examination. The final diagnosis was based upon microscopic sections.

Five groups of mice were used. Two were inbred strains which have been employed extensively in previous experiments. There were 100 mice from the agouti strain, No. 1194, and 80 mice from selected lines derived from Bagg albinos. A third lot of 23 mice were also Bagg stock but of different descent. They were grouped separately because they had not been tested for lung tumor susceptibility and turned out later to react differently in respect to skin tumors. A fourth group of 71 mice, pink-eyed, dilute brown in color, came from a very highly inbred strain, No. 62. The last group of 54 mice were hybrids of mixed ancestry but were uniform in being heterozygous for the dominant character hairless.

Strain 62 lived a much shorter time than the other 4 groups. By the 350th day no tumor-free mice were living so that no further change in tumor rate was possible. At this time 51.8 ± 4.5% of the mice which had survived the tarring had carcinoma. This rate was about the same as that of the hairless mice (54.7 ± 4.6%) but differed significantly from Strain 1194 (25.3 ± 3.0%) and the Bagg (a) group (17.1 ± 2.9%). The latter 2 do not differ significantly from each other. The small group of Bagg (b) stock were negative throughout the experiment. To make a mathematical comparison we may assume that if another animal had been included it would have been cancerous. On this basis the Bagg (b) mice would have a tumor incidence of 4.3% with a probable error of 2.9. This is significantly lower than the preceding rates.

The last 4 strains lived about twice as long as No. 62. By the end of the experiment the rate of the hairless had risen to 73.6 ± 4.1 %. Of the 1194 mice, 42.1 ± 3.4% had developed epitheliomas and of the Bagg (a) group 34.2 ± 3.7% had done so. The 2 latter rates do not differ significantly from each other but are different from the hairless and Bagg (b) groups. The rate of Strain 62 at 350 days was significantly greater than that ever attained by either Bagg group. Evidently there are marked differences in susceptibility to tar-induced skin tumors among these strains of mice.

Comparisons may be made as to the susceptibilities of different tissues. When the tar applications are distributed to a number of loci instead of being repeated on the same spot lung tumors rather than tumors of the skin are induced. For 3 of these stocks data are already available after using this technique. The animals were killed on the 300th day, thereby giving uniformity as to age. For the skin tumor experiment also the percentages are cited for the 300th day. The Bagg (a) group had a high incidence of lung tumors (92.8 ± 2.1%) and a low percentage of skin tumors (13.2 ± 2.6%). In Strain 62 the situation was reversed. There were only 12.9 ± 4.1% lung tumor and a higher incidence of skin tumor (51.8 ± 4.5%). Strain 1194 showed about the same degree of susceptibility to tumors in the 2 organs (22.4 ± 4.0 and 21.1 ± 2.8% respectively).

Data taken at the conclusion of the experiment in which the skin tumor technique was employed while not furnishing as strict a comparison provide additional evidence. The relationships here are similar to those already found except that in the 1194s the tumor incidence of the skin is now higher (42.1 ± 3.4%) than that of the lung (16.2 ± 2.9%). Both groups of Bagg mice have high lung tumor rates (81.5 ± 3.2 and 80.0 ± 6.0% respectively), and significantly lower skin tumor rates (34.2 ± 3.7 and 4.3 ± 2.9%). The members of Strain 62 did not live long enough to give information as to their lung tumor susceptibility but the hairless mice show a high incidence of both tumor types (73.6 ± 4.1% skin tumors and 73.8 ± 4.6% lung tumors). Evidently there is no correlation between the susceptibilities to tumor in the 2 organs. If these types are inherited separately there must be separate genes to represent them in the germplasm. More than one pair of Mendelian factors must be involved, though the exact number is undetermined.

7066 C

A Serological Difference Between Eastern and Western Equine Encephalomyelitis Virus.

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From cases of equine encephalomyelitis in New Jersey and Virginia a virus has been secured which closely resembles that found in a similar disease in the western United States by Meyer, Haring, and Howitt.[1]

Like the western virus the strains isolated from eastern cases are highly pathogenic for guinea pigs, less so for mice, and mildly virulent for rabbits. Tests on other animals and detailed studies on the routes of infection have not been made. Infection has been produced in guinea pigs by subcutaneous, intraperitoneal, and intracerebral injection; mice have been infected by these routes and also by intranasal injection. Rabbits given virus intracerebrally usually die, but if it is given intra- or subcutaneously they recover and are then immune to intracerebral injection. Their sera will neutralize the virus injected. The virulence of the eastern virus for laboratory animals appears to be greater than that of 2 western strains we have obtained from horse brains sent from Utah. It differs little in virulence from a Nevada and a South Dakota strain that have both been adapted to guinea pigs.

Strains of the western and the eastern virus have been compared serologically and a distinct difference has been found. In order to detect this difference it was necessary to test decreasing amounts of the virus (brain suspension from infected guinea pig) against undiluted serum. An example of a test is given in Table I. It will be seen that had a 10% brain suspension alone been used, neutralizing antibodies could not have been detected.

TABLE I.
Intracerebral Tests on Guinea Pigs.
Test injection: 0.1 cc. mixture of equal parts of virus and undiluted serum incubated 2 hours.

Virus		Serum		
No.	Dilution of 10% suspension	Rabbit immune to 121 Va.	Rabbit immune to 114 Va.	Normal rabbit (control)
121 Va.	Undiluted	Died in 76 hrs.	Died in 73 hrs.	Died in 74 hrs.
	10^{-2}	Lived	” ” 158 ”	” ” 90 ”
	10^{-4}	Died in 24 hrs. accident	Lived	” ” 99 ”
	10^{-6}	Lived	”	Lived
S. D.	Undiluted	Died in 95 hrs.	Died in 90 hrs.	Died in 65 hrs.
	10^{-2}	” ” 83 ”	” ” 98 ”	” ” 67 ”
	10^{-4}	” ” 85 ”	” ” 126 ”	” ” 99 ”
	10^{-6}	” ” 112 ”	” ” 108 ”	” ” 100 ”
		Rabbit immune to S.D.	Horse convalescent—East. Md.	Normal rabbit (control)
Nevada 1	Undiluted	Died in 216 hrs.	Died in 59 hrs.	Died in 85 hrs.
	10^{-2}	Lived	” ” 86 ”	” ” 92 ”
	10^{-4}	”	” ” 92 ”	” ” 128 ”
	10^{-6}	”	Lived	Lived

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[1] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, 74, 227.

After a series of tests, a dilution of 10^{-3} of a 10% brain suspension was selected as suitable for neutralization experiments. Each mixture of serum and virus was tested by intracerebral injection into 2 guinea pigs. The animals were etherized before the injection. A summary of the tests made is given in Table II.

TABLE II.
Summary of Neutralization Tests.

Immune sera		Eastern virus				Western virus			
Animals immune to virus from	No.	N.J. 109	Va. 114	Va. 119	Va. 121	Utah 3	Utah 4	Nev. 1	S.D.
Eastern United States	Rabbit 114 Va.	P	P	P	P	P-	No P	No P	No P
	Rabbit 121 Va.	P	?	P	P	P-	No P	No P	No P
	Horse 129 Va.	P	P	P	P	—	No P	No P	No P
	Horse 134 Va.	P	?	P	P	—	—	—	No P
	Horse 136 Va.	P	P	P	P	—	P	No P	No P
	Horse Md.	P	P	P	P	No P	—	No P	No P
Western United States	Rabbit Utah 3	No P	—	No P	No P	P	P	P	P
	Rabbit Nev. 1	No P	—	—	No P	P	P	—	—
	Rabbit S. D.	No P	No P	No P	No P	P	P	P	P
	Horse Nev.	No P	—	No P	No P	P	P	P	P
	Commercial antiserum Horse	No P	—	No P	No P	P	P	?	P-

P = Protection.
No P = No Protection.
P- = One of 2 guinea pigs survived.
? = Life prolonged but guinea pigs died.
— = No test made.

It is apparent that the western strains are serologically identical and that the eastern strains differ from them but not from each other. The fact that the difference between them is not merely one of virulence is shown by the failure to neutralize in both directions. Since the Nevada and South Dakota strains do not differ from the 2 freshly obtained Utah strains there is no reason to believe that their serological character has been modified by adaptation to guinea pigs.

The sera from the 4 eastern horses in the table were obtained from animals that had recovered from the disease. It will be noted that these sera neutralize all strains of the eastern virus. These results differ from those of Meyer, Haring, and Howitt,[1] who did not find neutralizing antibodies in the sera of recovered animals.

No explanation is offered at this time for the serological difference between the strains of virus from the 2 parts of the country. The clinical disease appears to be similar in both regions except that in the east its course is more rapid. The importance at present of the difference between the 2 strains lies in its bearing upon the preparation of immunizing agents or therapeutic sera.

7067 P

Swelling of Gelatin in a Series of Human Blood Sera.

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From the Gladwyne Research Laboratory, Gladwyne, Pennsylvania.

During the past thirty-five years, there has been considerable speculation as to the rôle played in pathology by abnormal increases and decreases in the state of swelling or water holding power of tissues.[1]

The resultant swelling tendency exerted upon tissue by plasma, or the lymph derived therefrom, is necessarily a function of the concentrations of a number of the plasma constituents. The writers believed that it would be desirable to have some single test available for this resultant tendency, and that such a test might be afforded by observation of the effect of plasma or serum upon some standard swelling material such as gelatin.

Small squares of Eastman Kodak Company's "ash-free" gelatin, weighing about .05 gm., were placed in Ringer-Tyrode solution (glucose omitted) and allowed to swell for 40 hours at about 10° C. The solution contained sodium bicarbonate, and was kept in contact with alveolar air. The pH was maintained within the range pH 7.2-pH 7.6. The percentage swelling of the squares was determined from a comparison of their swollen and original dry weights. It was of the order of 11 times in all cases, and, in any one series, the maximum difference between values was about 2 parts per hundred.

Blocks of gelatin were thus provided in a standard state of swelling. It was hoped that this state would be such that the blocks would swell further in some sera and shrink in others. This proved to be the case.

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[1] Ludlum, S. DeW., Taft, A. E., and Nugent, R. L., *Arch. Neurol. Psychiat.*, 1930, **23**, 1121.

In a series of 22 normal and pathological sera, the one showing the greatest swelling tendency caused a standard block to swell 5.1% further, and the one showing the least swelling tendency caused a 1.2% shrinkage. A standard block, as described above, was immersed in each serum for 20 hours in the ice chest, after which any change in weight was noted. The pH of each serum was adjusted by saturation with alveolar air and controlled between the limits pH 7.2-pH 7.8 throughout. Duplicate experiments indicated an unavoidable experimental variation of less than one percent.

The foregoing results indicated that, in a series of sera, the resultant swelling tendencies with regard to standard gelatin blocks may be measured and clearly differentiated. It next became of interest to determine the composition variable or variables primarily responsible for the swelling variations observed.

Series of synthetic solutions were prepared based upon Ringer-Tyrode solution containing 5% egg albumin and adjusted to pH 7.4-pH 7.6. In each series, the concentration of one factor was varied so as to include the maximum range of variation of this factor in pathological sera, and the effect of this variation upon the swelling of standard gelatin blocks was observed.

Preliminary experiments had shown that such variation of the calcium, potassium, glucose and urea concentrations caused swelling effects quite insufficient to account for the differences observed with the sera. Variation from pH 7 to pH 8 caused a maximum swelling difference of about one percent. Varying the total salt content from one-half the normal physiological value to one and a half times this value caused a swelling increase of only about 2%.

However, when the albumin content was varied from 2.5 to 10.0%, there was a clear cut swelling decrease about 8.0%, amply sufficient to account for the maximum difference of 6.3% observed with the series of sera, in which abnormal, widely variant protein contents were known to be present.

The foregoing experiments gave a strong indication that variation in the total protein contents were primarily responsible for the swelling differences observed with the series of sera. Refractive index data were available for the sera and were used as rough measures of their total protein content.[2] A comparison showed a definite increase in swelling tendency with decrease in refractive index, in entire agreement with the findings in the synthetic solution experiments.

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[2] Beltz, L., and Kaufman, E., *Z. Klin. Med.*, 1925, **101**, 409.

The first conclusion is that sera may be conveniently arranged in the order of their resultant swelling tendencies with respect to standard blocks of gelatin. The second is that the serum composition variable of primary importance in determining this resultant swelling tendency in any case is the total protein level. This effect of the protein level of the swelling solution upon the state of swelling of gelatin is predictable on the basis of recent findings of Northrop and Kunitz.[3]

7068

Observations on Coronary Occlusion.* II. Electrocardiographic Changes.

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In the course of experiments designed to study the effects of coronary artery ligation in dogs[1] it has been interesting to observe rather varied electrocardiographic changes and at this time a brief note is presented to describe some of the characteristic features observed.

Seventeen dogs were used. Under amytal anesthesia, with respirations maintained by positive pressure, the pericardium was exposed by a subperiosteal resection of the anterior portion of the fourth rib on the left. The anterior descending branch of the left coronary artery was ligated 1.5 cm. from its origin. Using conventional limb leads, electrocardiographic tracings were taken at hourly intervals for the first 12 hours, again at 24 hours, at daily intervals for the next week, and at weekly intervals thereafter. In each instance a control tracing was taken before the operation was begun, the animal being under full amytal anesthesia at the time. Two dogs served as controls. The operative procedures were duplicated in every detail except the actual tying of the ligatures about the coronary artery.

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[3] Northrop, J. H., and Kunitz, M., *J. Phys. Chem.*, 1931, **35**, 162.

* This investigation is supported by funds from the Josiah Macy, Jr., Foundation.

[1] Sutherland, F. A., Dial, D., and Harris, B. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1430.

The changes noted consisted of (A) disturbances of rhythm and (B) changes in direction and configuration of the T, ST and RST components of the electrocardiogram.

A. *Disturbances of Rhythm.* In 6 dogs ventricular fibrillation appeared as a terminal phenomenon within from 3 to 20 minutes following ligation. In a seventh dog ventricular fibrillation was noted approximately 23 hours after ligation. In this dog the ventricular fibrillation was transient, was followed successively by ectopic ventricular tachycardia, premature ventricular contractions, and finally by normal rhythm. Nodal and ventricular premature beats appeared in approximately half of the dogs at varying intervals after ligation—3½ to 24 hours—with the greatest incidence of onset from 6 to 8 hours after ligation. Usually within an hour or 2 after the onset of premature contractions, an irregular, ectopic ventricular tachycardia set in. The appearance of ectopic ventricular tachycardia usually presaged death of the animal within 24 to 48 hours. In 2 instances, however, the animals survived, the rhythm later reverting to normal.

B. *Changes in Direction and Configuration of T, ST and RST Components.* After ligation of the coronary artery varying changes in direction and configuration of T, ST and RST components were noted. These alterations were successive but not uniform in character. Within an hour after ligation there was a conspicuous increase in amplitude of the T waves which persisted for several hours. The most constant effect was an early cove-shaped negativity of the T waves; in most instances most marked in Leads I and II, but frequently present in all leads. These changes were noted within 1 hour following ligation, and varied from hour to hour thereafter. Early T negativity was usually followed later by increasing positivity, in some instances completely reversing the original changes. Progressive T changes continued to take place at a slower rate in the longer experiments but were present as late as 51 days following ligation. ST and RST segment deviations were less frequently noted, being present in only 5 instances. Contrary to the experience of Barnes and Mann[2] these deviations were not constant, 4 dogs exhibiting the T_1 type of RT deviation, one dog the T_3 type of RT deviation.

The control electrocardiographic tracings taken before operative procedures were begun indicate extreme variability of the "normal" T wave in the dog. Ten of 17 dogs exhibited T negativity of varying magnitude in Lead I usually accompanied by T negativity also in Lead II. The negative T waves were usually sharp, cove-shaped, not unlike that described as *typical of coronary closure.* In only one instance, however, was there any RST or ST deviation in these control tracings. In one of the 2 control animals no early electrocardiographic changes were noted. Nine hours after operation, however, there were slight directional changes in the T waves which became quite pronounced by the third, fourth and fifth days. Further slight directional changes were noted over a period of 3 weeks. In the second control animal marked T, ST and RST changes were noted in the first 9 hours post-operatively. Twenty-two hours after operation there were further directional changes in the T-waves and 2 days later the dog was found dead.

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[2] Barnes, A. R., and Mann, F. C., *Am. Heart J.*, 1932, **7**, 477.

7069 C

Destruction of Levo-, Dextro- and Racemic Hyoscine by Egg White and Rabbit's Serum.

W. F. VON OETTINGEN AND I. H. MARSHALL.

From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio.

It was demonstrated[1] that rabbit's serum destroys atropine (dl-hyoscyamine) and its isomers, levo- and dextro-hyoscyamine, at different rates and it was suggested that this accounts for quantitative differences of the systemic action of these alkaloids in different species. In the following report levo-, dextro- and racemic hyoscine* were studied as to their destruction by egg white and rabbit's serum.

The determination was performed in the same manner as described in the previous paper. In the first series 20 mg. of the alkaloids were dissolved in 2 cc. of water, mixed with 8 cc. of fresh egg white and incubated for 0, 3, and 6 hours at 38°C., three experiments being done for each compound and each series. At the end of the incubation period the alkaloids were isolated and determined by means of the Vitali reaction. It was found that with all 3 alkaloids without incubation all material added could be recovered within the limit of error which is ±5%; after 3 hours of incubation 21.9% were destroyed and after 6 hours 37.5%, the 3 isomers being destroyed at the same rate. It is interesting that under identical conditions atropine and its isomers were destroyed at the same ratio, namely ±5%, 21.5%, and 37%, the 3 isomeric forms showing also no differences in regard to their destruction.

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[1] von Oettingen, W. F., Marshall, I. H., *J. Pharm. Exp. Therap.*, in press.

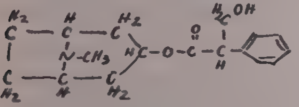
* The levo- and dextro-hyoscine used in this study were prepared by the Sandoz Chemical Works, Basle, Switzerland, and furnished through the courtesy of Dr. E. Rothlin in Basle.

Similar experiments were performed with rabbit's serum in the same proportion and with the precautions discussed in the previous paper. In this series also 3 experiments were made for each alkaloid and each time interval, which all gave identical results. After 6 hours 100% of the levo-, 68.3% of the dextro-, and 81% of the racemate were destroyed, which agrees with the values found in the atropine series, 100% for levo-, 57% for dextro-hyoscyamine and 81% for atropine (dl-hyoscyamine).

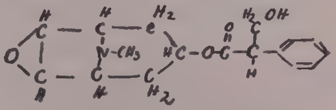
After an incubation period of 3 hours the destruction of the 3 alkaloids of both series was as follows:

d-Hyoscine	50%	d-Hyoscyamine	18%
l-Hyoscine	100%	l-Hyoscyamine	67%
dl-Hyoscine	79%	dl-Hyoscyamine	37%

It appears, therefore, that the hyoscine alkaloids are more rapidly destroyed in the beginning than the hyoscyamine preparations,



dl-Hyoscyamine (Atropine)



l-Hyoscine (Scopolamine)

As indicated by the formula for dl-hyoscyamine and l-hyoscine, both compounds have the tropic acid radical in common. According to Gadamer[2] the optical isomerism of these compounds is due to the stereoisomerism of the tropic acid radical, and the results reported in this paper and in our previous report on the destruction of both alkaloids and their isomers by egg white and rabbit's serum indicate also that the tropic acid radical is responsible for the different resistance of the optical isomers of the same rotation towards the decomposition by the serum.

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[2] Gadamer, *Arch. d. Pharm.*, 1901, **239**, 300, 663.

7070 P

Mucoid Bacterium Coli in Feces of Normal Subject.

LELAND W. PARR.

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Theobald Smith[1] was first to call attention to colon bacilli so similar in colony form to *B. aerogenes* as to be indistinguishable morphologically. Smith's culture "29" derived from a sample of spring water. Mucoid forms of colon bacilli have since been reported under a variety of conditions. Kuwaraba[2] reported a capsulated organism from panophthalmitis at first identified as Friedländer's bacillus, but later as *B. coli.* Revis[3] contaminated soil with feces and after several months obtained mucoid colon bacilli which at 20°C. produced slimy colonies as much as half an inch in diameter. These forms reverted readily to typical colon bacilli. Bordet and Ciuca[4] obtained mucoid "modified *B. coli*" by the action on the colon bacillus of a guinea pig peritoneal exudate produced by injecting *B. coli.* Gratia[5] confirmed the work of Bordet and Ciuca. He also produced non mucoid variants by aging a normal culture. Gory[6] obtained mucoid coli by the bacteriophagic action of filtered sewage. Theobald Smith and associates[7] in work on pathogenic *B. coli* from bovine sources (scours) dealt with mucoid forms and derivatives therefrom. Parr and Caldwell[8] recovered mucoid forms of various members of the colon-aerogenes group, including coli, from water samples from pumps near bored latrines. Lawson[9] reports the recovery of mucoid colon bacilli from diarrheal stools of infants. Dominick[10] encountered mucoid coli in well water regularly receiving chlorine. Furthermore the colon bacillus is ordinarily unencapsulated except by special technique, Churchman and Emelianoff.[11]

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[1] Smith, T., *Am. J. Med. Sci.*, 1895, **110**, 283.

[2] Kuwaraba, T., *Arch. f. Augenheilk.*, 1908, **60**, 323.

[3] Revis, C., *Centralbl. f. Bakt.*, II Abt., 1910, **26**, 161.

[4] Bordet, J., and Ciuca, M., *Compt. rend. Soc. Biol.*, 1920, **83**, 1293.

[5] Gratia, A., *J. Exp. Med.*, 1921, **34**, 115; 1922, **35**, 287.

[6] Gory, M., *Compt. rend. Soc. Biol.*, 1923, **88**, 49.

[7] Smith, T., *et al.*, *J. Exp. Med.*, 1927, **46**, 123, 133, 141, 155, 549.

[8] Parr, L. W., and Caldwell, E. L., unpublished data from the Research Laboratory, Alabama State Board of Health, Andalusia, Alabama.

[9] Lawson, G. M., 1933, personal communication, Louisville, Ky.

[10] Dominick, J. F., 1933, personal communication, Washington, D. C.

[11] Churchman, J. W., and Emelianoff, N. V., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 515

We here report the occurrence of encapsulated *B. coli,* giving rise to large, moist mucoid colonies on plate culture, derived from the feces of a normal, adult male. During 1932-33, 156 strains were isolated on Endo's agar from 16 fresh fecal suspensions in sterile saline in a preliminary attempt to determine the relative percentages of the different members of the colon-aerogenes group appearing on a medium on which all such members grow well. Unexpectedly it was found that on most platings all colonies were devoid of metallic sheen and were larger, more raised and more moist than the classical colon bacilli. A few times these mucoids were mixed on the plates with typical forms and once no mucoid types were encountered. Of the 156 isolations, 91% were *B. coli.* The mucoid colonies were made up of very short, encapsulated Gram negative bacilli which did not liquefy gelatin nor ferment saccharose, but which in all cases fermented dextrose and lactose with production of gas as well as acid were methyl-red positive, produced indol and failed to grow on citrate agar. No H_2S was produced. Motility was negative in 24 hr. broth cultures. Flagellar stains have not yet been made. Milk was on first isolation only acidified but on sub-culture coagulated. Broth suspensions remained homogenous. Serological investigations have not yet been undertaken. These forms have thus far maintained their mucoid nature despite manipulation designed to revert them to a non-encapsulated state.

The significance of these findings in a perfectly normal subject on an average mixed diet is manifold. Mucoid forms of most members of the colon-typhoid group are commonly considered variant strains produced only under unusual conditions and then rarely and temporarily. Further investigation may show them to be more common than is supposed. The close relationship of the Encapsulatus group to colon-aerogenes organisms is again emphasized from a new angle. From the work of Bordet and Ciuca, of Gratia, of Gory and of the Smith group such forms also relate to the problems of lysis, of the appearance of new races, of the inheritance of acquired characteristics and of virulence. And from the point of view of water bacteriology it must be said that if relatively stable mucoid forms of the colon bacilli may contaminate water all tests designed to hasten water analysis through colony differentiation of *B. coli* and *B. aerogenes* by differential plating procedures are to be questioned.

7071 P

Effect of Adrenalectomy on Salt Metabolism in Rats.

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The findings recorded were observed in studies to determine whether the adrenal glands regulated the metabolism of the body salts. White rats weighing between 150 and 180 gm. were used.

Salt balance studies were carried out on 4 pairs of animals before and after complete bilateral adrenalectomy. A control period of 5 to 6 days preceded the removal of the glands. Twenty-four hours after operation when the animals had completely recovered from the surgical shock, the study periods which varied from 6 to 11 days began.

The symptoms of adrenal insufficiency were: Loss of weight, decrease in food and water consumption, gradually progressing inactivity and weakness, and a diminution in the amount of urine and feces excreted. Death in the untreated animals occurred within 10 days after operation. Within 12 to 24 hours of death the respiratory rate increased, the body temperature fell, and there was a roughening of the fur with a rusty pigmentation of the hair about the head. Later, hemorrhages occurred about the nose and from the conjunctivae. Jumpiness and convulsions were noted terminally.

Balance studies were made for Ca, Mg, Na, K, P, Cl, and N. Our analyses show that during insufficiency there is not only a loss of sodium and chlorine as has been emphasized by other observers,[1] but a great loss of all elements studied—a negative balance for each of these occurred. The negative balance of N, P, K, Ca, and Mg, shows that tissue destruction has taken place.

Because of this loss from the body of these many basic elements following adrenalectomy, it occurred to us that in the treatment of adrenal insufficiency, one should aim at replacing all of those substances. To this end, after the animal was in marked insufficiency, a solution containing .0329% $CaCl_2$, .015% MgCl, .7% NaCl, and .0350% KCl, was substituted for the distilled water used in the control periods.

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[1] Marine, D., and Baumann, E. F., *Am. J. Physiol.*, 1927, 81; Loeb, R. F., *Science*, 1932, **70**, 420; Loeb, R. F., Atchley, D. W., Benedict, E. M., Leland, J., *J. Exp. Med.*, 1933, **57**, 775; Harrop, George A., Weinstein, Albert, Soffer, L. J., Trescher, J. H., *J. Am. Med. Assn.*, 1933, **100**, 1850; Harrop, George A., Soffer, L. J., Ellsworth, R., Trescher, J. H., *J. Exp. Med.*, 1933, **1**, 58.

The improvement in the animals following this change was striking. Even if this fluid was not given until within 2 to 3 hours before the time that death would have ordinarily occurred, the animals could be restored to a normal state within 4 to 5 hours after its administration. We have so far kept completely adrenalectomized animals in apparent health for 4 months on this mixture. No evidence of edema has appeared in these animals.

During severe insufficiency the animals were so weak that the salt mixture had to be given by pipette. Studies in progress suggest that other anions and cations should be included in the mixture, and possibly an increase in the amount of those ions already included. We have begun a comparative study using normal salt solution (.9% NaCl) as a treatment fluid. While our results are not as yet conclusive, it is our impression that these animals have not done as well as those on the salt mixture.

We do not feel certain that the action of adrenal hormone is primarily one of salt regulation, although this is quite probable. Further studies of the effect of starvation on the salt balance might elucidate this point. Such studies are now in progress. Our observations show that completely adrenalectomized animals may be kept in apparent normal health for long periods if sufficient salts are given.

7072 C

A New Form of Drop Recorder.

LAURENCE G. WESSON. (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt Medical School, Nashville.

Gibbs[1] classifies the drop recorders reported in the literature according to the essential mechanism as follows: (a) impulse, (b) weight, (c) expansion, (d) electrolytic, and (e) displacement. The simple and effective drop recorder here described depends upon a principle quite different from any described heretofore, and resulted because of our trial and abandonment of some of the most promising types, including that described by Gibbs.[2]

The essential part of our form of drop recorder consists of a short length of capillary tubing sealed to a bulb and T tube. A current of air from a pump or compressed air jet flows through the T tube and bulb and out of the capillary tube. The drops to be recorded as they fall from the drop inlet are blown through the capillary tube and momentarily hinder the passage of air sufficiently to create a slight increase of back-pressure that may be used to actuate a tambour or other recording device.

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[1] Gibbs, O. S., *J. Lab. Clin. Med.*, 1927, **12**, 686.

[2] Gibbs, O. S., *Science*, 1931, **74**, 549.

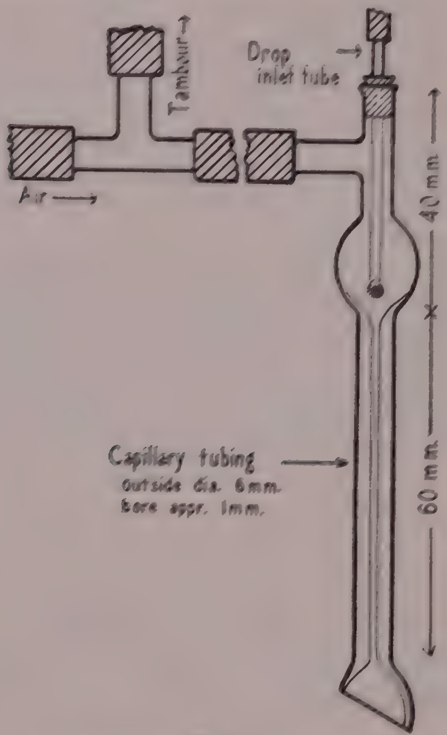

FIG. 1.
Drop recorder with approximate dimensions for convenient size.

The air pressure required is very slight, equivalent to a few centimeters of water, and requires no careful adjustment or maintenance of a constant pressure. The pressure required for the blowing out of the drops is so low that it is entirely compensated for by a few centimeters head of hydrostatic pressure in the drop inlet tube and ureteral or other cannula or catheter attached to the upper end of the drop inlet. If the size of the drops to be recorded is of importance, a change of the drop inlet tube, which may be a hypodermic needle of any gauge, a drawn-out glass tube, a suitable cannula, or a small metal tube, may be made without trouble. Drops as small as 0.01 cc. or as large as 0.05 cc. have been used with entire success. The degree of viscosity (for example, 15% gum acacia solution) or the character of the fluid (electrolyte content) are also unessential for the satisfactory operation of this form of drop recorder, so long as discrete drops may be obtained.

In physiological or pharmacological experiments, the drop inlet tube is attached at its upper end by small rubber tubing directly to the cannula, the flow from which is to be recorded. The recorder should be kept in an approximately vertical position so that the drops fall directly into the capillary tube without spreading over the walls of the surmounting bulb. To obtain a sufficiently rapid response of the tambour, the rubber dam should be taut. Certain response and recording may be easily obtained with this apparatus with as many as 360 drops per minute, in other words, with drops that nearly merge into a stream.

(This drop recorder may be had from E. Machlett and Son, 220 East 23rd Street, New York City.)

7073 C

Influence of Mineral Oil on Assimilation of Vitamin A from Spinach.

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The inhibitory effect of mineral oil on the absorption and utilization of vitamin A has been discussed by several workers.[1, 2, 3] Rowntree[1] considers the phenomenon of little significance so long as abundance of vitamin is provided. Jackson[2] finds but slight effect upon vitamin absorption if the mineral oil is fed separately from the butter fat. In none of the investigations has special attention been given to the effect of mineral oil upon vitamin A from a vegetable source, nor to the quantity of vitamin actually diverted by the parallel use of mineral oil.

Would mineral oil effect the carotin of plant cells in the same manner that it did the true vitamin A from an animal source? This question arose at the time some vitamin A assay work on a dry spinach product was being completed in the laboratory. At the end of the 8 weeks' test period these rats were given ½ cc. of mineral oil daily in addition to their spinach supplement. The results were striking and left no doubt that mineral oil does seriously interfere with the assimilation of the provitamin in plant cells.

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[1] Rowntree, J. I., *J. Nut.*, 1931, **3**, 345.

[2] Jackson, R. W., *J. Nut.*, 1931, **4**, 171.

[3] Dutcher, R. A., Ely, J. O., and Honeywell, H. E., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 953.

The subject seemed to warrant further consideration from a quantitative standpoint. Fresh green spinach was used in all subsequent experiments and the standard vitamin A technique discussed by Sherman[4] was employed. Repeated control experiments had demonstrated that 10 mg. of spinach contained slightly more than one unit of vitamin A. When quantities of mineral oil ranging from ¼ to 1 cc. were fed with 10 mg. of spinach, the rats failed to gain and died in from 3 to 11 weeks. This was to be expected as all workers have emphasized that the effect of mineral oil is most pronounced when minimum or marginal doses of the vitamin supplement are employed.

The present question may then be stated: How much more spinach would it be necessary to feed in the presence of ½ cc. of mineral oil in order to obtain the same limited growth as that obtained with 10 mg. of spinach alone?

Graded amounts of spinach were fed, keeping the mineral oil dosage constant (½ cc. daily). This amount has been used by several investigators and is comparable to a moderate human dose. The grade of oil used was that known as Standard Oil "Stanolind" heavy (Viscosity 310 sec. at 100° F. and pour test 0°F.).

All rats fed 10, 20 and 40 mg. of spinach daily as the sole source of vitamin A plus ½ cc. of oil died in a few weeks with definite symptoms of vitamin A deficiency. Those fed 80 mg. of spinach plus the oil showed temporary gains in some cases with subsequent loss of weight and death in 8 out of 11 cases. The 3 rats which gained were in an experimental group fed during the spring on home-grown spinach which proved to have a slightly higher A content than that shipped in during the winter. A limited number of tests using larger quantities of spinach (as a source of A) indicated on the average that 100-120 mg. of spinach daily would be necessary to promote a unit gain of 3 gm. per week if fed with ½ cc. of mineral oil.

When the same spinach and mineral oil supplements were fed separately and at least 6 hours apart the 80 mg. of spinach permitted slightly more than unit growth, or approximately the same as that obtained with 10 mg. of spinach when no mineral oil was fed.

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[4] Sherman, H. C., Chemistry of Food and Nutrition. Macmillan, 1932, 361.

Thus 10-12 times as much spinach was required to furnish a unit of vitamin A when accompanied by ½ cc. of mineral oil; and about 8 times as much when fed separately at least 6 hours apart. It would seem that the effect of mineral oil on vitamin A assimilation is of sufficient magnitude to be significant.

TABLE I.

Average gain or loss of weight in rats receiving graded quantities of fresh spinach as the sole source of vitamin A with and without mineral oil.

Spinach** mg.	Mineral Oil cc.	No. Rats	Total Change in wt. gm. aver.		Survival* Week's Average
			Gain	Loss	
10		9	+31		8+
20		4	+64		8+
80		7	+79		8+
20	1	3		—15	5
40	1	3		—25	3
40	1/2	4		—26	3.5
80	1/2	11	+27(3)	—23(8)	8 6,6
80	1/2 P.M.	4	+45		8+
80 P.M.	1/2	9	+27		8+
120	1/2	2	+ 3		5+
120 P.M.	1/2	4	+26		5+

*The total experimental period was 8 weeks except in the last 2 groups where it was limited to 5 weeks.

**Fed in A.M. unless otherwise indicated.

Higher melting point paraffins have not been investigated as to their effect on the assimilation of vitamin A. They have, however, been recommended by some physicians as more efficient laxative agents than the fluid oils. Paramels* are a product of this type containing an oil of viscosity 200 sec. at 100°F. The final product has a variable melting point between 84 and 104°F. Paramels were fed to rats in doses of 0.65 gm. daily, equivalent to ½ cc. of mineral oil and under identically the same conditions as reported above.

Rats fed 80 mg. of spinach plus 0.65 gm. of paramels grew on the average of 4½ gm. a week or 1½ units, as compared with loss of weight and death in 8 out of 11 rats fed the equivalent doses of ½ cc. of fluid mineral oil.

Thus it would appear that the higher melting point paraffin oil and wax mixture has a less detrimental effect upon vitamin A assimilation than does the fluid mineral oil.

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* Produced by Battle Creek Food Co., Battle Creek, Mich.

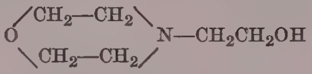
7074 C

Comparative Pharmacology of Some Thiomorpholine Derivatives.

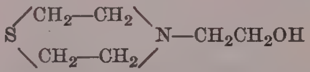
DAVID I. MACHT.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Maryland.

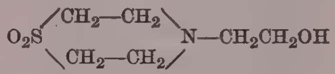
Gardner and Haenni[1] described a series of compounds containing the morpholine rings, which they claimed exhibited some local anesthetic activity. The general structure of their compounds is expressed by the formula,



Burrows,[2] under the direction of Professor E. Emmet Reid in the Laboratory for Organic Chemistry, Johns Hopkins University, prepared 2 series of analogous compounds, which the writer had the privilege of investigating pharmacologically. One series was composed of thiomorpholines. The first of these, labelled No. 0, is thiomorpholine ethanol and has the following structure,



In addition, 7 esters were prepared from this alcohol, namely, the acetate, propionate, butyrate, valerate, caproate, heptoate and benzoate. In this paper these are labelled Nos. 1 to 7, respectively. A second series of compounds, analogous to the thiomorpholines and containing 2 oxygen atoms attached to the sulphur, was also prepared. These may be termed sulphoxy-thiomorpholines, the first member of the series, sulphoxy-thiomorpholine ethanol, being labelled No. Ox; and the 7 other members, esters similar to those of the thiomorpholines, being labelled 1x to 7x, respectively. The structure of the first member of this series is as follows:



The action of aqueous solutions of these 2 series of compounds was studied on living seedlings of *Lupinus albus,* on tadpoles of *Rana pipiens,* on goldfish, *Carassius auratus,* and on cats under ether anesthesia.

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[1] Gardner and Haenni, *J. Am. Chem. Soc.,* 1931, **53**, 2763.

[2] Burrows and Reid, in press.

Effect on Plants. Seedlings of *Lupinus albus* were grown in plant-physiological solutions containing the drugs in concentrations of 1:500. The effect is shown in Table I. Both series of compounds inhibited the growth of the plants, the least toxic being Nos. O and Ox. The sulphoxy-thiomorpholines were in every case more toxic than the plain thiomorpholine compounds.

TABLE I.
Effect of thiomorpholine derivatives on growth of lupinus albus seedlings.

Thiomorpholine S 1:500 Concentration of	Phytotoxic Index	Thiomorpholine SO_2 1:500 Concentration of	Phytotoxic Index
Compound No. 0	87%	Compound No. 0x	59%
" " 1	77%	" " 1x	28%
" " 2	75%	" " 2x	38%
" " 3	64%	" " 3x	29%
" " 4	69%	" " 4x	33%
" " 5	51%	" " 5x	27%
" " 6	23%	" " 6x	21%
" " 7	59%	" " 7x	28%

Effect on Tadpoles. Tadpoles of *Rana pipiens,* 4 weeks old, were placed in solutions of the drugs, 1:1,000 and 1:500. The results obtained in the weaker solution were as follows:

Tadpoles in Solution 1:1,000

Thiomorpholine	No. 0	1	2	3	4	5	6	7
Killing time, hrs.	48	24	15	12	10	4½	3½	15

The toxicity increased progressively from compound 0 to ester 6. Compound 7, or the benzoic acid ester, however, was less toxic than some other aliphatic esters.

Toxicity for Goldfish. Small goldfish, 2 in. long, were placed in solutions of 1:250 of both series of compounds. Time of onset of muscular incoordination, indicated by floating on the side, and the time required to effect respiratory paralysis were both noted. The results are exhibited in Table II. Here again the esters were pro-

TABLE II.
Goldfish (Carassius Auratus) in solution 1:250

Thiomorpholine	0	1	2	3	4	5	6	7
Floating on side in	2 hr.	37 min.	39 min.	20 min.	9 min.	5 min.	4 min.	2 min.
Respiration paralyzed in	24 "	64 "	73 "	30 "	25 "	18 "	9 "	4 "
Sulphoxy-Thiomorpholine	0x	1x	2x	3x	4x	5x	6x	7x
Floating on side in	12 min.	4 min.	3 min.	13 min.	7 min.	3 min.	2 min.	4 min.
Respiration paralyzed in	24 "	13 "	16 "	22 "	14 "	10 "	5 "	7 "

gressively more toxic than the mother substance, or thiomorpholine ethanol. The sulphoxy compounds were all more toxic than the plain thiomorpholine compounds.

Anesthetic Effect. Solutions of both series of compounds varying from 0.1 to 1.0%, were tested for local anesthetic action on rabbits' eyes and frogskin. Contrary to the results obtained by Gardner and Haenni with morpholines, it was found that the thiomorpholines had very little local anesthetic effect, the only one producing definite local anesthesia being compound No. 7, or the benzoic acid ester. One per cent solutions of this ester, applied to the rabbit's eye, produce local anesthesia lasting from 5 to 10 minutes. None of the sulphoxy-thiomorpholines was found to produce any local anesthetic action.

Experiments on Blood Pressure and Respiration. Experiments were made with intravenous injections of the aqueous solutions of thiomorpholines in cats kept under ether anesthesia. When injected in doses of from 10 to 15 mg. per kg. weight of cat, it was found that all the compounds produced an immediate fall in blood pressure and a slight inhibition of the respiration. The depressant action on the blood pressure increased progressively from compound 0 to compound 7. This depressant effect was of short duration, however, and the circulation became normal after the lapse of a few minutes. The most depressant action on the respiration was exerted by compound 7. The sulphoxy-thiomorpholines acted in much the same way but were slightly more toxic.

Summary. 1. The pharmacology of thiomorpholine ethanol and 7 esters thereof was compared with that of sulphoxy-thiomorpholine ethanol and 7 esters thereof in respect to local anesthetic effect, toxicity and action on blood pressure and respiration. 2. Only the benzoic ester of thiomorpholine-ethanol showed any appreciable local anesthetic effect, and none of the sulphoxy-thiomorpholine compounds revealed any definite local anesthetic action. 3. The toxicity of both series of compounds is not very great but the sulphoxy-thiomorpholine derivatives are in general more toxic than the thiomorpholine derivatives.

7075 C

Occurrence of Cataract in Experimental Pancreatic Diabetes.*

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Despite the fact that the existence of "diabetic cataract" as a distinct clinical entity has been questioned by some investigators,[1, 2, 3, 4] cataractous changes in the eyes of diabetic patients are not uncommon. Spalding and Curtis[2] reported the presence of cataract in 10% of Joslin's diabetic subjects while Von Noorden[5] found such lens changes in 13% of his diabetic cases. The age at which cataracts appeared in these patients is obviously of importance in determining the significance of these percentages, for cataractous changes of some degree occur frequently in normal subjects during late-middle and old age. In none of the cases reported by Spalding and Curtis[2] did cataracts occur earlier than 53 years of age. Twenty-eight of Von Noorden's 62 diabetic patients with cataracts, however, were under the age of 50. The presence of cataracts in young adults and in children suffering from diabetes is of more significance in establishing a relation between these 2 conditions, for lenticular changes other than congenital cataract are rare in normal individuals of these age periods. Although cataracts have been reported in diabetic children[6-11] their occurrence in juvenile diabetes is not common. In a careful survey of the diabetic patients studied in Joslin's clinic from 1898 to 1931 White[10] reported 15

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* The expense of this investigation was defrayed in part by a grant from the Research Board of the University of California, Berkeley. The insulin was generously donated by the Eli Lilly Company.

[1] Gallus, E., *Deutsch. Med. Wchnschr.*, 1919, **45**, 658.

[2] Spalding, F. M., and Curtis, W. S., *Boston Med. and Surg. J.*, 1927, **197**, 165.

[3] Gradle, H. S., *Indiana State Med. Assn. J.*, 1929, **22**, 2.

[4] Jess, A., *Kurzes Handbuch der Ophthalmologie, bearbeitet von W. Gilbert, A. Jess, H. Ronne, F. Schieck.* 1930, Julius Springer, Berlin, 253.

[5] Von Noorden, C., *Die Zuckerkrankheit und ihre Behandlung.* 1917, Julius Springer, Berlin, 305.

[6] Sherrill, J. W., *J. Metab. Res.*, 1922, **1**, 667.

[7] Strouse, S., and Gradle, H., *J. Am. Med. Assn.*, 1924, **82**, 546.

[8] Major, R. H., and Curran, E. J., *J. Am. Med. Assn.*, 1925, **84**, 674.

[9] Joslin, E. P., Treatment of Diabetes Mellitus, 1928, Lea and Febiger, Philadelphia, 833.

[10] White, Priscilla, Diabetes in Childhood and Adolescence, 1932, Lea & Febiger, Philadelphia.

[11] Joslin, E. P., *Southern Med. J.*, 1933, **26**, 1.

TABLE I.

Dog	Date of Birth	Date Depancreatized	Duration pancreatectomy to eye examination		Condition of Lens	
			Yr.	Mo.	Right	Left
1 Whte-Chrms Male diabetic		Dec. 25, 1930	2	8½	Peripheral striations.	Peripheral striations.
2 Airdle Female diabetic	Nov. 20, 1930 *	Sept. 1, 1931	2	½	Faint opacity at posterior pole and beneath posterior capsule.	Faint opacity at posterior pole and beneath posterior capsule
3 Airdle Female Normal	Nov. 20, 1930 *				Normal	Normal
4 Brn-c-Wht-Col Female diabetic		June 29,1932	1	2½	Diffuse striated opacification involving anterior and posterior cortex; moderate sclerosis of nucleus.	Striations in spoke formation.
5 Red-a-Wht Female diabetic	Nov. 29, 1931 **	Sept. 5, 1932	1	½	Normal	Normal
6 Blk-a-Wht Male diabetic	Nov. 29, 1931 **	Sept. 1, 1932	1	½	Small triangular opacity beneath anterior capsule.	Small triangular opacity beneath anterior capsule.
7 Brownie Female diabetic		March 11, 1931	2	6	Faint peripheral striations; generalized spotting; numerous vacuoles scattered throughout whole lens.	Changes similar to that found in right eye but less marked.

8 Wht-c-Brn-Hd Female diabetic	Sept. 16, 1931 ***	July 27, 1932	1	1⅔	Diffuse striations irregularly distributed throughout whole lens.	Peripheral striations.
9 Brn-a-Blk Female diabetic	Sept. 16, 1931 ***	Aug. 1, 1932	1	1½	Normal	Normal
10 Blk Female normal	Sept. 16, 1931 ***				”	”
11 Hyn Female diabetic		March 2, 1931	2	6½	Irregular opacity of posterior cortex.	Similar to right eye.
12 Bty Female diabetic		April 2, 1931	2	5½	Peripheral striations.	Several long dense striations extending from periphery to center; several vacuoles.

Three groups of litter mates are included in this table: Dogs 2 and 3; Dogs 5 and 6; Dogs 8, 9, 10—indicated by *, **, ***, respectively.

cases with cataractous lenses among 750 subjects who developed diabetes in childhood, and 4 cases showing similar lens pathology among 251 patients in whom the onset of the disease occurred in adolescence.

In the present investigation a study was made of the eyes of completely depancreatized dogs kept alive with insulin. This animal is particularly well adapted for studies of this nature, for in addition to providing an animal with pancreatic diabetes in which the diagnosis is never in question, such animals can be kept under well-controlled conditions.

The animals recorded as diabetic are completely depancreatized dogs that have been maintained in this laboratory in good nutritional condition for long periods of time by means of a suitable diet and insulin. Each dog received twice daily, at 8:00 a. m. and at 4:00 p. m., a diet consisting of 225 gm. of lean meat, 70 gm. of sucrose, and 5 gm. of bone ash. Vitamin B concentrate[12] was added to the diet at least 3 times per week. Vitamins A and D were supplied as the unsaponifiable portion of cod liver oil or as raw cod liver oil. The animals were injected with 8 units of insulin at each time of feeding. The 24 hour sample of urine of the animals always contained glucose, in varying amounts from day to day.

The results are shown in tabular form. They indicate quite definitely that a high incidence of cataract is present in completely depancreatized dogs maintained for long periods of time in apparently good nutritional condition by means of diet and insulin. Eight out of the 10 diabetic dogs showed lens changes varying in degree from peripheral striations to diffuse irregular opacification. All cataracts found in these animals were either incipient or immature. As judged by ophthalmoscopic examination, the lens changes were similar to those found in early senile cataract of human subjects.

The results suggest that the duration of diabetes is a factor in the development of cataract by depancreatized dogs. Five of these animals were diabetic for 2 years or more. All showed marked lens changes. Five other animals were diabetic for 14.5 months or less and it is interesting to note that the 2 depancreatized dogs which had normal lenses were diabetic for approximately one year only, the shortest period of any of the animals studied. The duration of the diabetes, however, does not determine the extent of the cataractous process for dog 4 that had been diabetic for 14 months at the time of its eye examination had marked lens pathology, whereas dog 2 that had been depancreatized 2 years prior to its eye examination revealed only peripheral striations in both lenses.

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[12] **Evans, H. M., and Lepkovsky, S., *J. Nutrit.*, 1931, 3, 353.**

Old age as a factor in the production of the cataractous changes in the depancreatized dogs studied can be ruled out for lens opacities were discovered in animals 2 years old or younger (dogs 6 and 8). As will be pointed out below, cataracts were not found in young normal dogs.

A large number of normal dogs of various ages were examined with respect to lens pathology. Two normal dogs, litter mates of dogs that had been depancreatized, are included in the table. Dog 3, a normal dog, was placed on the diet recorded above on September 1, 1931, the day when its litter mate, dog 2, had been depancreatized. Thus both normal and depancreatized dogs have been kept under the same environmental and dietary conditions for the same length of time. Dogs 8, 9, and 10 are also litter mates, the first 2 being diabetic and dog 10, normal. The normal control of this group has also been maintained under the same dietary and environmental conditions as its 2 diabetic litter mates. The lenses in dogs 3 and 10 were found normal. Seventy normal dogs in various laboratories of the Medical School were also examined. Only 4 of these animals showed cataractous involvement. It is significant, moreover, that the latter were advanced in years, all showing signs of old age.

7076 P

Ovogenesis in the Ewe and Cow.

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The evidence presented by Allen[1] did much to establish the concept that ovogenesis extends into the postpubertal period in mammals. He found that the germinal epithelium contributed new ova in the adult mouse and, further, that the rate of ovogenesis varied according to the stage of the oestrous cycle. The greatest number of mitotic figures was found in the germinal epithelium during oestrus. Allen and coworkers[2] found in the sow that there was an elimination of most of the larger follicles in the ovaries coincident with the maturation of a set of follicles. Engle,[3] working with the mouse, found a greater number of both small and large follicles in active stages of degeneration during oestrus and early metoestrum than at other stages, although the actual differences found were relatively small. In a study of ovogenesis and follicular atresia in the rat, guinea pig, dog, cat, and man Evans and Swezy[4] concluded that the maturation of one or more follicles involved the destruction of all remaining follicles and that after ovulation a new ovogenetic wave began.

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[1] Allen, E., *Am. J. Anat.*, 1923, **31**, 439.

[2] Allen, E., Kountz, W. B., and Francis, B. F., *Am. J. Anat.*, 1925, **34**, 445.

Twenty-five pairs of ewe ovaries and a slightly smaller number of cow ovaries, removed at various phases of the oestrous cycle, have been carefully studied. From some of these complete series; from others only a few sections were made.

No evidence has been found which would indicate that the new ova arise from the germinal epithelium in the sexually mature ewe or cow. Rather, it appears that they arise in the neogenic layer beneath the ovarian tunic in a manner very similar to that described by Simpkins[5] for the human. As clear-cut evidence has been presented by Allen[1] in the mouse and by Evans and Swezy[4] in the rat, guinea pig, dog, and cat that ova do arise from the germinal epithelium in mature animals, it would appear that species or group differences exist in this respect.

Healthy and atretic follicles are found in the ovaries at all times in approximately the same proportions. If there are differences it will be necessary to make actual counts to determine them. Small differences in atresia such as Engle found in the mouse might easily have been overlooked. Marked differences in the number of atretic follicles in the ovaries of different individuals removed during the same stage of the cycle do exist. This has been found to be true for all species of mammals thus far studied. There is no indication that the maturation of one or more follicles involves the destruction of all remaining follicles. During oestrus and early metoestrum follicles of considerable size are found in an apparently healthy condition. Thus, in the species under consideration, it seems unnecessary to assume that the life span of an ovum is limited to an interval of time corresponding to the length of the cycle. In fact, the appearance of healthy follicles during oestrus, fully one-half their mature size, makes it appear entirely probable that the development of a definitive ovum takes place over a period corresponding to the length of 2 or more cycles as Hammond[6] has postulated. It is likely that overemphasis has been placed upon the rhythmicity of ovogenesis and follicular atresia in mammals in general. Although Allen clearly showed that there were distinct cyclical differences in the rate of mitoses in the germinal epithelium, the evidence favors the view that the production and destruction of follicles are continuous processes not entirely limited to single phases of the cycle. The presence of follicles in practically all intermediate stages of development is the rule. Further, very little evidence exists in favor of the view that ovogenesis and atresia are initiated by factors which initiate and regulate the oestrous cycle. Changes in hormonal concentrations affect them, no doubt, to a greater or lesser degree dependent upon the species. Ovogenesis and atresia occur, however, during the prepubertal period and continue after hypophysectomy.

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[3] Engle, E. T., *Am. J. Anat.*, 1927, **39**, 187.

[4] Evans, H. M., and Swezy, Olive, *Mem. Univ. of Cal.*, 1931, **9**, 119.

[5] Simpkins, C. S., *Am. J. Anat.*, 1932, **51**, 465.

7077

Vaccination of Monkeys Against Pneumococci with Special Reference to Oral Immunization.*

JOHN A. KOLMER AND ANNA M. RULE.

From the Research Institute of Cutaneous Medicine of Philadelphia.

Cecil and Steffen have found the Philippine macaque *(Macacus syrichtus)* more suitable than *Macacus rhesus* for the production of experimental pneumococcus pneumonia but the former were not available; consequently we were obliged to use the latter.

Four monkeys were given a subcutaneous injection of 1 cc. of vaccine per kilo of weight carrying 20 million Type I and an equal number of Type II and Type III pneumococci in broth suspension sterilized with 0.5% tricresol every 5 days for 6 doses.

Four additional animals were given 1 cc. of acid-killed mixed vaccine of the same numerical strength per kilo of weight by stomach tube every 5 days for 6 doses.

Two weeks after the last dose 2 monkeys of each series, with a normal control, were given intratracheal injections of 0.5 cc. of 1:20 dilutions of 8 hour broth culture of Type I pneumococcus. At

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[6] Hammond, J., Physiology of Reproduction in the Cow. Cambridge Univ. Press, 1927, 44.

* Aided by a grant from the Samuel F. Fels Fund for Research.

the same time the remaining 3 monkeys of each series, with a normal control, were given intratracheal injections of 0.5 cc. of 1:20 dilution of 8 hour broth cultures of virulent Type II pneumococcus.

The rectal temperature of each animal was recorded twice daily, with daily leukocyte counts.

None of the animals succumbed and none developed septicemia (positive blood cultures) but both controls became very sick within 24 hours after the intratracheal inoculations, with total leukocytes reaching 24,900 and 33,500 per cmm. respectively and rectal temperatures 104 to 105.2°F. subsiding to normal (about 101-102°F.) within 3 to 6 days.

The 2 monkeys given the vaccine subcutaneously and inoculated intratracheally with Type I remained perfectly well, the highest rectal temperature being approximately 103°F. on the second day after inoculation with 18,200 leukocytes per cmm. of blood. Approximately the same results were observed with the 2 monkeys given the vaccine by stomach tube and inoculated intratracheally with Type I pneumococcus.

However, the 2 monkeys given the vaccine subcutaneously and the 2 given the vaccine by stomach tube and inoculated intratracheally with Type II pneumococcus became quite sick and ran a course of fever and leukocytosis quite similar to the normal controls.

With *Macacus rhesus* monkeys it has been found difficult therefore to compare the immunizing capacity of the 2 vaccines but we believe that subcutaneous injections of the chemically killed vaccine proved superior to the oral administration of the acid-killed vaccine, and that both were more effective against Type I than against Type II pneumococcus.

Summary. In general terms the immunization of *Macacus rhesus* monkeys with subcutaneous injections of a mixed chemically killed vaccine proved superior to the oral administration of an acid-killed vaccine of the same strength and dosage. Vaccination against Type I pneumococcus was more effective than against Type II with both vaccines.

7078

Vaccination of Rabbits Against Pneumococci with Special Reference to Oral Immunization.*

JOHN A. KOLMER AND ANNA M. RULE.

From the Research Institute of Cutaneous Medicine of Philadelphia.

The use of pneumococcus vaccines by *oral* administration is largely based upon the investigations of Ross[1] and in an article on prophylactic immunization of rabbits against pneumococcus pneumonia and septicemia Kolmer and Rule[2] found that 7 daily doses of vaccines by oral administration engendered a high degree of immunity against Type I pneumococcus, somewhat less against Type II and still less against Type III, as determined by the intratracheal injection of virulent cultures of these types. Kolmer and Amano[3] also observed that rabbits may be effectually immunized against meningitis and septicemia following intratympanal and intracisternal injections of virulent Types I, II and III pneumococci, by the oral administration of vaccines given once a day for a week. Particularly encouraging results were observed with HCl acid-killed, sodium taurocholate-dissolved and heat-killed milk cultures of virulent pneumococci and it was recommended that when time permits, humans with pneumococcus paranasal sinusitis and otitis be prepared for operative procedures by the oral immunization of autogenous vaccines as a possible protection against extension of the infection to the meninges.

These investigations were continued with rabbits to determine: (1) dosage of vaccine by oral administration in relation to acquired immunity; (2) duration of immunity after oral immunization; (3) to compare the immunity response from subcutaneous injections with oral administration of the vaccines.

Since "acid-killed" pneumococcus vaccines appeared to engender the highest degree of immunity in rabbits[2] they were mostly employed. The organisms were cultivated in a broth medium for 24 hours and sufficient N/1 hydrochloric acid added to give an N/15 concentration. After standing for 2 hours at room temperatures the

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* Aided by a grant from the Samuel F. Fels Fund for Research.

[1] Ross, V., *J. Exp. Med.*, 1930, **51**, 585; *Immunol.*, 1926, **12**, 219, 237; *J. Lab. and Clin. Med.*, 1927, **12**, 566; PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 273.

[2] Kolmer, J. A., and Rule, A. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 107.

[3] Kolmer, J. A., and Amano, K. W., *The Laryngoscope*, August, 1932.

vaccines were subcultured for sterility. Numerically they averaged about 500 million per cc. The dosage was 0.2, 0.5, 1.0, 2.0, and 5.0 cc. per kilo administered daily for 7 doses by stomach tube. Numerically the dosage varied therefore from approximately 100 to 2500 million per kilo of weight. One week after the last dose each animal, including normal controls, was given intratracheal injections of living broth cultures. Immediately or within a few hours after death the heart blood was cultured for pneumococci and complete necropsies performed with special reference to the lungs.

Dosage in Relation to Acquired Immunity of Rabbits.—Table I summarizes the results of an experiment with Type I pneumococcus. Rather large doses were required for engendering immunity amounting approximately to 7 doses of 2 cc. of 24 hour broth cultures, or approximately 1000 million acid-killed cocci per kilo of weight.

TABLE I.
Dosage in relation to oral immunization of rabbits against Type I Pneumococcus Pneumonia and Septicemia.

Dosage* per Kilo. cc.	No. Immunized	Survived**	Died***	% Survivals
0.2	8	4	4	50
0.5	8	4	4	50
1.0	8	6	2	75
2.0	8	7	1	88
5.0	8	8	0	100
Controls not Immunized	8	0	8	0

* Seven at daily intervals by stomach tube.
** After intratracheal inoculation with 0.5 cc. of 1:30 dilution of 6 hour broth culture given one week after last dose of vaccine.
*** All with positive heart blood cultures.

All of the rabbits succumbing died in from 3 to 6 days after intratracheal inoculation with living highly virulent culture and all showed positive heart blood cultures. However, even doses as small as 0.2 cc. of vaccine per kilo produced a rather high degree of immunity in 50% of the animals while a dose of 1.0 cc. per kilo immunized 75%, 2.0 cc. about 88%, and 5.0 cc. 100% of rabbits. (Table I.)

With vaccines of Type II and Type III pneumococci the results were less definite. (Table II.)

Both strains were less virulent for rabbits than Type I since about half of the inoculated normal controls survived. But the results have shown that acid-killed vaccines by oral administration engendered immunity in doses varying from 0.5 to 5.0 cc. per kilo, the

TABLE II.

Dosage in relation to oral immunization of rabbits against Type I and II Pneumococcus Pneumonia and Septicemia.

Dosage* per Kilo. cc.	Type II				Type III			
	No. Immunized	Survived **	Died	% Survivals	No. Immunized	Survived ***	Died	% Survivals
0.2	4	2	2	50	5	2	4	40
0.5	4	3	1	75	5	3	2	60
1.0	4	3	1	75	5	4	1	80
2.0	4	4	0	100	5	5	0	100
5.0	4	4	0	100	5	5	0	100
Controls not Immunized	4	2	2	50	5	3	2	60

* Seven at daily intervals by stomach tube.

** After intratracheal inoculation with 2 cc. of a 6 hour broth culture given one week after the last dose of vaccine.

*** After intratracheal inoculation with 2.5 cc. of a 6 hour broth culture given one week after the last dose of vaccine.

2.0 cc. dose per kilo being approximately the minimal dosage when given daily for 7 consecutive days. Group IV pneumococci were not included.

Number of Doses in Relation to Acquired Immunity in Rabbits. Rabbits were given 1, 2, 3, 5, and 7 daily consecutive doses of 2 cc. of the Type I vaccine by stomach tube. Seven days after the last dose, each, along with normal controls, was inoculated intratracheally. As shown in table, 3, 1, 2 and 3 doses were without appreciable effect while 5 daily doses were approximately the minimum required for completely effective immunization.

TABLE III.

Number of doses of Type I Pneumococcus Vaccine by oral administration in relation to acquired immunity of rabbits to Pneumococcus Septicemia and Pneumonia.

No. Doses*	No. Immunized	Survived**	Died	% Survivals
1	8	1	7	12
2	8	0	8	0
3	8	0	8	0
5	8	7	1	88
7	8	8	0	100
Controls not immunized	8	0	8	0

* 2.0 cc. each by stomach tube.

** After intratracheal inoculation with 0.5 cc. of 1:30 dilution of 6 hour broth culture given one week later.

Duration of Immunity After Oral Immunization of Rabbits. We expressed the opinion[2] that immunity of rabbits after 7 daily consecutive doses of Type I vaccine administered by stomach tube, may last 4 months or longer. This would appear to be about the longest duration in experiments of this character, remembering that some immunity may be present which is masked by the rather severe test following intratracheal inoculation of sufficient amounts of living culture to produce the death of all normal controls within 72 hours. Rabbits given 5 daily consecutive doses of acid-killed vaccine of 2.0 cc. by stomach tube, developed a rapid immunity as shown by the survival of all when inoculated intratracheally one week later. Four weeks later this immunity was appreciably reduced, and it was apparently gone, in the terms of the severe experiment, within 6 months as shown by the results with Type I vaccine summarized in Table IV.

Subcutaneous vs. Oral Immunization of Rabbits. Two vaccines were employed. The first for subcutaneous injection was an unheated broth suspension containing approximately 20 million of Type I and an equal number of Type II and Type III pneumococcus per cc. and sterilized with 0.5% tricresol. The second for oral administration contained the same numbers of the 3 strains killed in an N/15 concentration of hydrochloric acid. Both were administered in a dose of 1 cc. per kilo but the results have indicated that the doses were too small, since our own work reported herein has shown that larger doses are required and the same has been apparently observed by Cecil and Steffen[4] in their work on the vaccination of monkeys against pneumococcus pneumonia.

TABLE IV.
Duration of Immunity of rabbits to Type I Pneumococcus following 5 daily doses of 2.0 cc. of Vaccine by oral administration.

Interval after Immunization*	No. Immunized	Survived**	Died	% Survivals
1 week	6	6	0	100
4 "	6	5	1	83
8 "	6	4	1	66
4 mo.	6	2	4	33
6 "	6	0	6	0
Controls not immunized	6	0	6	0

* With 5 daily doses of 2.0 c.c. by stomach tube.
** After intratracheal inoculation with 0.5 cc. of 1:30 dilution of 6 hour broth culture.

Twelve rabbits were given a subcutaneous injection of the first vaccine in dose of 1 cc. per kilo every 5 days for 6 doses. One week later 4 were inoculated intratracheally with living Type II and 2 survived (50%) and the remaining 4 were inoculated with a virulent Type III with 1 survival (25%).

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[4] Cecil, R. L., and Steffen, G. I., *J. Exp. Med.*, 1921, **34**, 245; 1923, **38**, 149.

These results are summarized in Table V. Rabbits vaccinated with chemically killed mixed pneumococcus vaccine by subcutaneous injection yielded a better immunity response than rabbits vaccinated with equal numbers of an acid-killed pneumococcus vaccine by stomach tube administration in the same dose per kilo of weight. Better results would probably have been observed had larger doses been given but these experiments were finished before we realized the importance of this dosage factor. However, the results are not without value as indicating the superior immunizing capacity of subcutaneous injections of vaccine.

Summary. 1. Vaccination of rabbits against Types I, II and III pneumococcus by oral administration of acid-killed vaccines requires rather large doses amounting approximately to 1000 million per kilo of weight, although smaller doses (100 million per kilo) were sometimes effective, especially in the case of Type I pneumococcus. 2. One dose of 1000 million Type I acid-killed vaccine by oral administration sometimes produced effective immunization but best results were observed with a minimum of 5 doses at daily intervals. 3. Pneumococcus immunity after oral administration of Type I acid-killed vaccine is of short duration as determined by survival after intratracheal injections of virulent culture. One month after vaccination the degree of immunity is decreased, and practically extinct at the end of 6 months. 4. The subcutaneous injection of rabbits with a chemically killed vaccine containing a total of 60 million of Types I, II and III per cc. gave a better immunity response than an acid-killed vaccine of the same strength by oral administration in dose of 1 cc. per kilo of weight.

TABLE V.
Subcutaneous versus oral immunization of rabbits with mixed Types I, II and III Pneumococcus Vaccines.

Route of Immunization	No. Immunized	% Survivals after Intratracheal Inoculation With Type I	With Type II	With Type III
Subcutaneous	12	75	50	25
Oral	12	50	0	0
Controls (12) not immunized	—	0	0	0

7079 P

Methylene Blue, Nitrites, and Sodium Thiosulphate against Cyanide Poisoning.

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Following the reports of Sahlin,[1] Eddy,[2] Brooks,[3] Geiger,[4] Hanzlik,[5] Hug,[6] and Wendel,[7] we investigated the comparative antidotal action of methylene blue and amyl nitrite in cyanide poisoning and found that methylene blue detoxified 2 M.L.D.'s and amyl nitrite by inhalation 4 M.L.D.'s of sodium cyanide.[8] Amyl nitrite is thus twice as effective as methylene blue. To date we have studied other nitrites and sodium thiosulphate. The last compound has been shown to have an antagonistic action against cyanide by several investigators.[9–13] The results summarized in Table I were all obtained in dogs. It should be noted that nitroglycerine is ineffective as an antidote; while sodium thiosulphate detoxifies 3 M.L.D.'s and so-

TABLE I.

Drug	No. M.L.D.'s of NaCN Detoxified
Nitroglycerine	0
Methylene Blue	2
$Na_2S_2O_3$	3
$C_5H_{11}NO_2$	4
$NaNO_2$	4
$C_5H_{11}NO_2$ and $Na_2S_2O_3$	10
$NaNO_2$ and $Na_2S_2O_3$	13

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[1] Sahlin, B., *Skandinav. Arch. Phys.*, 1926, **47**, 284.

[2] Eddy, N. B., *J. Pharm. Exp. Ther.*, 1931, **41**, 449.

[3] Brooks, M. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1228; *Am. J. Phys.*, 1932, **102**, 145.

[4] Geiger, J. C., *J. Am. Med. Assn.*, 1932, **99**, 1944.

[5] Hanzlik, P. J., *J. Am. Med. Assn.*, 1933, **100**, 357.

[6] Hug, E., *Compt. rend. soc. biol.*, 1932, **111**, 519; 1933, **112**, 511.

[7] Wendel, W. B., *J. Am. Med. Assn.*, 1933, **100**, 1054; *J. Biol. Chem.*, 1933, **100**, Proc. c.

[8] Chen, K. K., Rose, C. L., and Clowes, G. H. A., *J. Am. Med. Assn.*, 1933, **100**, 1920.

[9] Lang, S., *Arch. Exp. Path. Pharm.*, 1895, **36**, 75.

[10] Heymans, J. F., and Masoin, P., *Arch. internat. pharmacodyn. thér.*, 1897, **3**, 359.

[11] Hunt, R., *Arch. internat. pharmacodyn. thér.*, 1904, **12**, 447.

[12] Voegtlin, C., Johnson, J. M., and Dyer, H. A., *J. Pharm. Exp. Ther.*, 1926, **27**, 467.

[13] Turner, B. B., and Hulpieu, H. R., *J. Pharm. Exp. Ther.*, 1933, **48**, 445.

dium nitrite 4 M.L.D.'s of sodium cyanide. A more striking antidotal effect is exhibited when amyl or sodium nitrite is supplemented with sodium thiosulphate. The combination of the nitrite and thiosulphate does not only show synergistic action but surpasses the sum of their individual values. For example, the number of fatal doses detoxified by sodium nitrite and sodium thiosulphate is 13 instead of 7. It is a definite case of potentiation. Compared with methylene blue, the coupling of sodium nitrite and sodium thiosulphate is 6½times as effective. The following protocol is representative of the experiments:

Dog, female, weighing 22 kg., received NaCN subcutaneously at 1:50 p. m., 78 mg. per kg. (13 M.L.D.'s); 1:51 p. m., received intravenously $NaNO_2$ 22.5 mg. per kg. and $Na_2S_2O_3$ 2 gm. per kg.; 2:00-2:22 p. m. vomited 5 times; 2:23 p. m. drank water; 3:10 p. m. received intravenously $NaNO_2$ 10 mg. per kg. and $Na_2S_2O_3$ 0.5 gm. per kg.; 3:16 p. m. vomited; 4:53, 7:57, and 9:50 p. m. received intravenously $NaNO_2$ 10 mg. per kg. and $Na_2S_2O_3$ 0.5 gm. per kg.; 8:15 a. m. of the next day, completely recovered.

To test the crucial efficacy of the nitrite-thiosulphate therapy, dogs were given large amounts of cyanide, say 10 M.L.D.'s, and allowed to develop the cardinal signs of cyanide poisoning such as convulsions, loss of corneal reflex, and failing respiration. As long as there was gasping, the intravenous administration of sodium nitrite and sodium thiosulphate enabled the animals to breath briskly at once, stand up within a few minutes, and recover completely in several hours.

Hug[14] showed that dogs receiving 6 M.L.D.'s of HCN and rabbits receiving 5 M.L.D.'s of KCN were saved with $NaNO_2$ and $Na_2S_2O_3$. Buzzo and Carratalá[15] also reported that rabbits could tolerate 18 M.L.D's of KCN if $NaNO_2$ and $Na_2S_2O_3$ were injected after the poison; whereas $NaNO_2$ alone detoxified 3 M.L.D.'s, and $Na_2S_2O_3$ alone 10 M.L.D.'s of KCN. The synergistic action of the nitrite and thiosulphate appears to be beyond doubt.

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[14] Hug, E., *Prensa méd. Argent.*, 1933, **20**.

[15] Buzzo, A., and Carratalá, R. E., *Seman. méd.*, 1933, **40**, 1966.

7080 P

Potentiation of Antidotal Action of Sodium Tetrathionate and Sodium Nitrite in Cyanide Poisoning.

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In a previous communication we[1] reported the synergism and potentiation of the antidotal action of sodium or amyl nitrite and sodium thiosulphate against cyanide poisoning. The combination of sodium thiosulphate with sodium nitrite proves to be better than with amyl nitrite. It becomes interesting to ascertain whether or not sodium tetrathionate will similarly potentiate the detoxifying action of sodium nitrite, since the tetrathionate has been shown to reduce the toxicity of hydrocyanic acid by Foresti[2] and Draize.[3] In a series of experiments with dogs our results reveal exactly the same synergism and potentiation that occur with sodium thiosulphate.

With the combination of sodium nitrite and sodium tetrathionate,* at least 3 dogs out of groups of 5 survived 13 or less M.L.D.'s of sodium cyanide; whereas, with sodium nitrite alone only 4 M.L.D's and with sodium tetrathionate alone 3 M.L.D.'s of NaCN were detoxified. The antidotal effect of sodium nitrite and sodium tetrathionate given together thus exceeds the sum of those contributed individually by sodium nitrite and sodium tetrathionate. It is therefore another case of potentiation of action.

The nitrite-tetrathionate combination is apparently efficacious in the late stages of cyanide poisoning. Dogs receiving large doses of NaCN have completely recovered at the point of respiratory failure. The following protocol can be taken as an example of our experiments:

Dog, male, weighed 13.2 kg.
10:59 a. m.—p. (pulse rate) 112, r. (respiratory rate) 48.
11:04—injected subcutaneously 72 mg. of NaCN per kg. of body weight (12 M.L.D.'s).
11:10—clonic convulsions and labored breathing.

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[1] Chen, K. K., Rose, C. L., and Clowes, G. H. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 250.

[2] Foresti, B., *L'Ateneo parmense*, 1931, **3**, No. 6, through *Revist. Sud-Amer.*, 1932, **15**, 628.

[3] Draize, J. H., *Science*, 1933, **78**, 145.

* A part of the sodium tetrathionate used in this investigation was kindly supplied by Dr. O. A. Beath, the Agricultural Experimental Station, University of Wyoming, to whom we acknowledge our indebtedness.

11:12—corneal reflex disappeared and respiration suddenly ceased. The animal was at once injected intravenously $NaNO_2$ 22.5 mg. per kg., followed by $Na_2S_4O_6$ 500 mg. per kg.
11:15—p. 68, r. 12; corneal reflex returned.
11:16—p. 108, r. 92.
11:25—p. 166, r. 132 (panting).
11:31—raised its head.
12:14 p. m.—injected intravenously $NaNO_2$ 10 mg. per kg. and $Na_2S_4O_6$ 125 mg. per kg.
12:23—up on its feet.
12:25—p. 156, r. 80.
1:18—p. 160, r. 20.
2:05—drank water.
4:45—ate food, urinated.
8:00 a. m. next day—no obvious untoward effects.

We have also studied the combination of methylene blue and sodium tetrathionate or thiosulphate in cyanide poisoning, and observed a synergistic action. The results are, however, much less striking.

7081 C

Glycogen Formation after Oral Administration of Mannitol to White Rats.

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Glycogen formation is stated to occur after the oral administration of mannose to animals, but the conversion of mannitol, the hexahydric alcohol which may be obtained by reduction of the aldehyde group of mannose, to glucose or glycogen has not been definitely proven. It has been maintained that sorbitol,[1] the hexahydric alcohol derived from glucose, may alleviate the hypoglycemic symptoms in rabbits which have received insulin and when administered to diabetic patients may increase the respiratory quotient. Rosenfeld[2] was unable to observe any significant increases in liver glycogen after feeding mannitol to dogs. Pflüger[3] in a critical evaluation of the data of Külz concluded that the increased glycogen deposition obtained by this investigator was within the limits of experimental error.

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[1] Reinwein, H., *Deut. Arch. klin. Med.*, 1929, **164**, 61.

[2] Rosenfeld, G., *Centr. inner Med.*, 1900, **21**, 177.

[3] Pflüger, E. F. W., *Das Glykogen und seine Beziehungen zur Zuckerkrankheit*, Bonn, 1905, 2nd Ed., 215.

We have studied the glycogen content of the liver after the oral administration of *d*-mannitol by the method of Cori. Young white rats were fasted 24 hours and fed either 2 or 4 cc. of a 15% solution of mannitol by stomach tube. The amount of mannitol fed was determined by passing the mannitol solution through the stomach tube into an evaporating dish, which was then placed in an oven and dried to constant weight. After absorption periods of 2, 3, 4, and 6 hours, the rats were killed and the glycogen content of the liver was determined as described previously.[4]

TABLE I.

Glycogen content of liver of white rats after oral administration of Mannitol.

Wt. after fast gm.	Absorption period hr.	Mannitol fed mg.	Liver Glycogen %
143	2	214	0.07
160	2½	214	0.05
137	3	214	0.08
129	3	214	0.12
130	4	250	0.16
135	4	250	0.08
110	6	505	0.19
114	6	505	0.05
115	6	505	0.07
120	6	505	0.08
123	6	505	0.06
132	6	505	0.09
136	6	505	0.07
145	6	505	0.07

Average value for 9 control animals **0.05 (range 0.02-0.06).**

The results are presented in Table I. The glycogen values for the livers of a control series of 9 rats which received 2 cc. of water after 24 hour fasting periods varied from 0.02 to 0.06% with an average of 0.05%. Inspection of the data of the table fails to reveal any significant increases in liver glycogen after oral administration of mannitol as compared with the values obtained for the control series.

After these experiments were completed, Carr and his co-workers[5] reported a similar study of the glycogen content of the liver of white rats, previously fasted and then fed a mixture of cacao-butter and mannitol (33%) over a period of 80 hours. The glycogen content of the liver of the rats fed mannitol and cacao-butter ranged from 0.62 to 1.80% with an average value of 1.23%, as compared with the average glycogen content of the livers of control rats fed cacao-butter alone of 0.14%.

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[4] Catron, L. F., and Lewis, H. B., *J. Biol. Chem.*, 1929, **84**, 553.

[5] Carr, C. J., Musser, R., Schmidt, J. E., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, **102**, 721.

It is impossible to compare the results of this series with our own experiments since the treatment of the animals was quite different in the 2 series. Under the experimental conditions used by us, it is evident that mannitol did not serve as a ready available source of glycogen. A similar lack of ready utilization of mannitol is shown by the reported failure of mannitol to relieve insulin shock in white rats.[6]

7082 C

Concentration of Poliomyelitis Virus by Ultrafiltration.*

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The obvious desirability of finding a means for the more adequate concentration of any of the filterable viruses, led us to attempt to adapt the ultrafiltration method, used by Seibert[1] in the study of tuberculin, to the concentration of poliomyelitis virus. That several of the well studied filterable viruses can be retained by filters or ultrafilters has been known for some time. But the work has been done largely for the purpose of determining the size of the viruses. Krueger and Schultz[2] and Clifton, Schultz and Gebhardt[3, 4] for example, have made careful studies of the filterability of poliomyelitis virus through graded collodion membranes. Their results indicate that the size of this virus is certainly less than 50 millimicrons in diameter and probably below 25 millimicrons. We have previously employed distillation *in vacuo* and fractional protein precipitation (Clark, Schindler and Roberts[5] and Clark, Roberts and Preston[6]) for the successful purification and concentration of poliomyelitis virus. Both of these methods, however, necessitate the subsequent elimination of the large amounts of salts by dialysis, before the material can be used for animal inoculation. The method here presented avoids that difficulty and we hope that it may give a more adequate basis for the study of the properties of poliomyelitis and other viruses.

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[6] Voegtlin, C., Dunn, E. R., and Thompson, J. W., *J. Pharmacol. and Exp. Therap.*, 1925, **25**, 168.

* Aided by a grant from the Milbank International Committee for the study of Infantile Paralysis and later by a grant from the National Research Council.

[1] Seibert, F. B., *J. Biol. Chem.*, 1928, **78**, 345.

[2] Krueger, A. P., and Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 600.

[3] Clifton, C. E., Schultz, E. W., and Gebhardt, L. R., *J. Bact.*, 1931, **22**, 7.

[4] Poliomyelitis. 1932, p. 49. Milbank International Committee for the Study of Infantile Paralysis.

Dried soluble cotton† was dissolved in concentrations of 8, 10, and 12% in glacial acetic acid and allowed to age, as a rule, at least a month, before being used to form the membranes over the alundum shells of medium porosity. Sterile distilled water was employed for washing the membranes, subsequently testing for the presence of acid with Congo Red. At least a liter of water was run through the membrane after the test for acid became negative. The permeability of the membranes was determined by their capacity to hold back Congo Red, a solution of crystallized egg albumin and a solution of hemoglobin. The Congo Red was tested for by the addition of acid to the ultrafiltrate, and the presence or absence of egg albumin was determined by using a specific precipitating serum of high titre. The increasing density of the red color in the "concentrate," when a small amount of laked blood had been added to the material to be filtered, and the colorless character of the ultrafiltrate served as an indicator of value. After our technic had been controlled in this manner a number of times, we discontinued the addition of any of these foreign substances to our filtrates. Careful visual inspection, the rate of flow of the wash water, and the gradual concentration in the ultraresidue of the small amount of hemoglobin normally present in brain cord filtrates proved ordinarily to be sufficient checks of the adequacy of the membranes.

In our earlier experiments, we autoclaved the membranes after they had been washed. This made them less permeable and at the same time more fragile but nevertheless, successful results were obtained by this method. Both the 8% and 10% autoclaved membranes proved adequate for the concentration of poliomyelitis virus; the 12% membranes became too impermeable. Several experiments with this procedure are presented in Table I.

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[5] Clark, P. F., Schindler, J., and Roberts, D. J., *J. Bact.*, 1930, **20**, 213.

[6] Clark, P. F., Roberts, D. J., and Preston, W. S., *J. Prev. Med.*, 1932, **6**, 47.

† From Charles Cooper and Company, New York.

TABLE I.*

Experiment 1

Virus concentrated with 8% Autoclaved Membrane.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results
212	Ultraresidue	2	14-1	Paralyzed in 12 days Typical Polio.
213	"	0.2	14-1	Remained Well
214	Ultrafiltrate	2	—	" "
215	"	0.1	—	" "
216	Filtrate	0.4	—	" "

* All injections made under ether anesthesia. Animals held under daily examination for at least a month.
Rockefeller mixed strains of poliomyelitis virus used throughout.
All injections of amounts under 1 cc. made in 1 cc. volumes except Exp. 1 in which 2 cc. volumes were used.
Diagnoses controlled by microscopic examination of sections.

Experiment 2

Virus concentrated with 10% Autoclaved Membrane.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results
201	Ultraresidue	1	32-1	Paralyzed in 15 days Typical Polio.
241	"	.05	32-1	" " 11 " " "
230	Ultrafiltrate	1	—	Remained Well
231	"	.05	—	" "

Experiment 4.

Virus concentrated with 8% Autoclaved Membrane.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results
250	5% Paper Filt.	2	—	Remained Well
257	" "	.1	—	" "
254	Ultraresidue	2	54-1	Paralyzed in 7 days Typical Polio.
255	"	.1	54-1	Remained Well
259	Ultrafiltrate	2	—	" "

The great difficulty in handling the brittle autoclaved membranes led, however, to a change in the method. Beginning with the sterile glacial acetic acid solutions of cotton, aseptic procedures were employed throughout. Cylindrical suction flasks were specially blown, each barely large enough to admit a single shell covered with its membrane and so small as to leave a residue of only 5 to 15 cc. at the end of the filtration process. As reservoirs for sterile distilled water for washing the membranes, large suction flasks were provided with two hole rubber stoppers, one hole for an outlet glass tube and the other for a receiving brass funnel which had a screw top which could be made air tight with sterile vaseline. A battery of such small ultrafiltration flasks can be set up each with its own reservoir and attached to a common suction pump. In practice the whole apparatus is assembled and autoclaved with all its connecting tubes and clamps before coating the alundum shells with the cellulose solution. The shells are then removed from the covered vessel in which they have been autoclaved, using sterile cotton gloves in the handling, since rubber gloves are difficult to sterilize and were a source of contamination earlier in the process. The shells are hermetically sealed into the suction flasks with a hot sealing mixture consisting of 3 parts resin and 2 parts beeswax. Care must be used to have the flasks full of fluid so that the membranes shall be kept wet; otherwise, bubbles will be formed with so high a surface tension as to make filtration impossible with an ordinary water pump. After the washing has been completed, the virus filtrate is introduced through the brass funnel into the same reservoir and the ultrafiltration actually begun. The ultrafiltrate comes over with increasing slowness as the membranes become coated with the somewhat viscous residue. When large amounts have been in process of filtration requiring several days for completion, we have usually protected the virus by immersing the suction flasks in a refrigerating system of cold running water.

This method has been fairly satisfactory. Many details must be

TABLE II.*
Experiment 9.
Virus concentrated with 10% unautoclaved membranes.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
341	Filtrate	1	—	Paralyzed in 11 days	Typical Polio.
342	"	.1	—	Remained Well	
343	"	.01	—	" "	
345	Ultraresidue	.1	20-1	Paralyzed in 8 days	" "
346	"	.01	20-1	Remained Well	

Experiment 11.
Virus concentrated with 10% unautoclaved membranes.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
342	Ultraresidue	.0001	165-1	Remained Well	
344	"	.001	165-1	Paralyzed in 6 days	Typical Polio.
356	"	.01	165-1	" " 6 "	" "
353	"	.1	165-1	" " 6 "	" "
357	Filtrate	.001	—	Remained Well	
343	"	.01	—	Paralyzed in 15 days	" "
347	"	.1	—	" " 8 "	" "
352	Ultrafiltrate	2	—	" " 11 "	" "

*Details of injections, the same as in Table 1.

controlled, but, with care, sterile concentrates can be obtained. Not only are hemoglobin and egg albumin held back by 8% and 10% membranes, but also the virus of poliomyelitis may be concentrated by this method as indicated by representative experiments in Table II. In only 2 out of many series did the virus pass the membranes of 8% concentration or higher. One of these was an experiment in which the membranes were sterilized in 70% alcohol in order to avoid the difficulties of the autoclaved membranes. It is well known that alcohol increases the permeability of such membranes. The other instance of virus passage (Table II. Experiment 11) was probably due to some unobserved break in the membranes, since we have repeatedly controlled the ultrafiltrate, even to the extent of concentrating it by distillation *in vacuo* and injecting this concentrated ultrafiltrate into monkeys without obtaining an infective dose of the virus. In testing these "concentrates" by the only known method, injection into susceptible monkeys, we find that although increase in potency is demonstrable, the infectivity does not increase proportionately to the actual decrease in the volume. In Table II, Experiment 9, for example, the virus filtrate was concentrated 20 times by volume, but the ultraresidue was only 10 times as effective as the original filtrate in producing complete paralysis in monkeys. In Experiment 11, the virus filtrate was concentrated 165 times by volume, but was still only 10 times as potent as the original virus. Doubtless considerable virus is adsorbed on the membrane in the process of filtration, but it would appear probable that we also have in these experiments indication of the concentration of an inhibiting factor in poliomyelitis brain cord suspensions, as suggested by Leiner and v. Wiesner[7] and studied more completely by Jungeblut.[8]

Conclusions. A method for the ultrafiltration and concentration of poliomyelitis virus is described. This should be applicable to the further study of the properties of poliomyelitis and other filterable viruses. The method does not result in a direct proportional volumetric increase in the potency of the ultraresidue. A concentration of an inhibiting agent is suggested.

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[7] Leiner and v. Wiesner, *Wien. Kl. Woch.*, 1910, **23**, 817.

[8] Jungeblut, C. W., *J. Immun.*, 1932, **22**, 99.

7083 P

On the Prophylactic and Therapeutic Value of Specific Immune Serum in Experimental Poliomyelitis.

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Comparatively few studies have been reported on the protective and therapeutic value of immune serum in experimental poliomyelitis in monkeys. The results vary somewhat, but indicate in the main that specific immune serum, while of doubtful therapeutic value, is of definite prophylactic value. We report observations on *M. rhesus* monkeys injected with immune serum either shortly before or at varying periods of time after inoculation with virus, the latter being administered in most of the experiments by the intranasal route,* the normal route of infection in man. The method of inoculating the experimental animals with virus by the intranasal route has already been reported by us.[1] The method described has yielded infection in about 95% of animals so inoculated. The immune serum employed in most of the experiments was one produced by injecting a horse with poliomyelitis virus over a period of nearly 2 years, at the end of which time its potency was such that 1 cc. of the serum contained sufficient immune substance to inactivate *in vitro* at least 25,000 minimum infecting doses of virus.[2]

Thus far we have carried out 11 experiments on the prophylactic value of immune serum, involving a total of 18 controls and 59 test animals. Convalescent monkey serum was employed in 2 of the experiments and immune horse serum in the remainder. The serums were administered by different routes; the amounts injected varying from a total of 1.75 cc. in one of the experiments to more than 30 cc. in other experiments. In most instances the serum was administered 24 hours prior to the inoculation of the animal with virus.

The results obtained in the individual experiments vary considerably, the percent survivors in the serum inoculated group ranging from 0% in one experiment (1.75 cc. of serum intracranially) to

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* All procedures, including intranasal inoculation, carried out under ether anesthesia.

[1] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1010.

[2] Schultz, E. W., and Gebhardt, L. P., *J. Immunol.*, in press.

100% in 3 of the experiments. Out of the total number of 58 serum treated animals 20 (or 34%) failed to develop the disease, while among the 18 controls all but one (or 94%) developed poliomyelitis. The average protection is somewhat below that realized in more limited studies by other investigators. Our results do not lead us to believe that pooled convalescent monkey serum is any more or any less effective as a prophylactic agent than the immune horse serum.

The experiments designed to test the *therapeutic* value of immune serum were carried out in much the same manner as those dealing with its prophylactic value, except that the serum injections in the therapeutic series were given 24 or more hours after inoculating the monkeys with virus. Nine experiments, in which a total of 12 controls and 32 test monkeys were employed, have been carried out in this part of our study. In most of these experiments the initial dose of serum was administered within 2 days following the inoculation of virus. The serum was administered by different routes and the amount injected was generally large in comparison with the amount usually administered per unit of body weight in the serum treatment of poliomyelitis in man. This applies also to poliomyelitis convalescent monkey serum, employed in 4 of the experiments.

The results give no indications whatever that serum is of any value 2 or more days after the animals have been successfully inoculated with virus. All of the serum treated animals, save one, developed the disease in about the same length of time and with about the same amount paralysis as the controls. The animal which failed to develop the disease had received 20 cc. of immune horse serum within 24 hours after its inoculation with virus. A companion monkey similarly treated developed the disease.

Our results indicate that once the virus has become implanted in the nervous tissue immune serum therapy is without appreciable effect. They, moreover, suggest that the entrenchment of the virus in the tissues occurs sometime within 2 days after the virus is instilled into the nasal passages of the experimental animal. Should these facts apply also in the natural infection of man, as they may, it would then appear highly improbable that immune serum, whatever its antibody content may be, can be of any appreciable value in combatting poliomyelitis unless it is administered sufficiently in advance of an exposure to the virus to forestall its implantation in the nervous system and at the same time not too far in advance of an exposure to the virus for the passive protection to wear off. This leaves to immune serum a very limited field of usefulness in the control of poliomyelitis.

7084

Effect of Hypophysectomy on the Ovary of Immature Rats.

HANS SELYE. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

We know that the immature gonad is largely independent of the pituitary. Malformations of the pituitary, such as those associated with anencephaly, do not influence the embryonic development of the ovary, although they inhibit the growth of the adrenal cortex very markedly.[1] It has also been shown that the anterior pituitary-like hormone is unable to produce follicle maturation and formation of corpora lutea in the ovary of the very young rat; its only effect at an age of 6-12 days is luteinization of theca cells.[2] It seemed of interest, therefore, to study the effect of hypophysectomy on immature rats. In previous experiments we saw that the immature

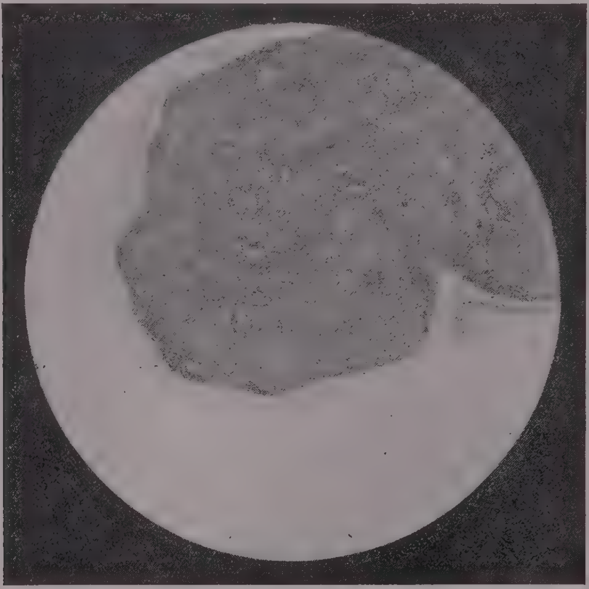

FIG. 1.
Low magnification of an ovary taken from a rat hypophysectomized at 18 days of age, and killed 25 days after operation. Picture shows that there is considerable follicular atresia, but many follicles are still normal.

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[1] Jaffe, R., and Tennenberg, I., *Hirsch's Handbuch. d. Inneren Sekretion,* **1**, 520.

[2] Selye, Collip and Thomson, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 647.

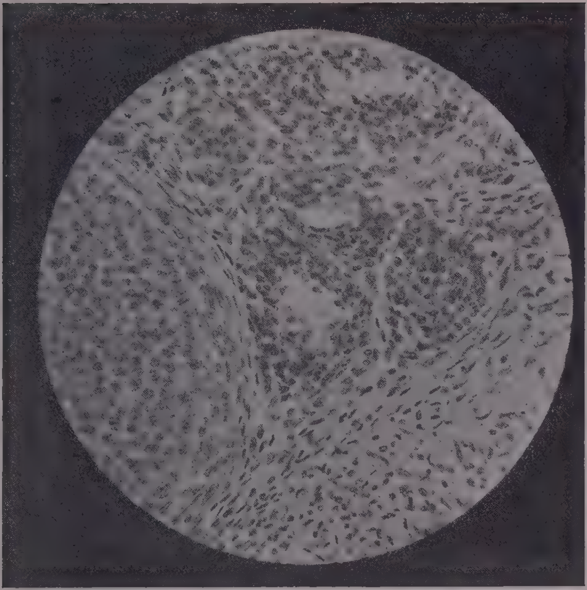

FIG. 2.

High magnification of a part of the ovary shown in Fig. 1. To the left, normal follicle surrounded by normal theca cells; in the center and to the right 2 atretic follicles the theca cells of which show definite signs of hormone deficiency (typical thecal deficiency-cells).

follicles of young rats may persist a very long time after the removal of the pituitary, and although the follicular atresia in such animals is usually considerable the results of hypophysectomy are not very clear-cut because the number of follicles undergoing atresia is also very considerable in the normal immature rat. We thought that our recent observations[3] on the effect of hypophysectomy on the theca might be used to detect more obvious signs of pituitary deficiency in the immature rodent. We hypophysectomized 20 female rats 18 days of age and killed them at various intervals between the 10th and 25th days after operation. They all showed both normal and atretic follicles, and many typical thecal deficiency cells as described previously.[3] The chromatin of their nuclei aggregates in large, dark granules, leaving unstained spaces in between (Figs. 1 and 2).

It is interesting to note in this connection that the transformation of thecal cells into deficiency cells occurs only around the atretic follicles, while the theca of normal follicles maintains its normal

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[6] Selye, Collip and Thomson, *Endocrinology*, 1933, **17**, 494.

appearance (Fig. 2). The reason for this phenomenon is not known to us, but it seems that the living granulosa and ovum protect the theca. This observation reminds one of the fact that thecal luteinization as produced by the anterior pituitary-like hormone of pregnancy urine also appears most readily in thecal cells which are not in immediate contact with living granulosa cells.

We conclude from this experiment that the hypophysis has a definite trophic influence on the ovary of the rat long before the animal reaches maturity. It is interesting to note that in these immature rats the most obvious signs of deficiency after hypophysectomy appear in the theca—that is, in those cells which are the only ones which respond to the administration of A.P.L. in the very young rodent.

7085

Effect of Anterior Pituitary-like Hormone on the Ovary of the Hypophysectomized Mouse.

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From the Department of Biochemistry, McGill University, Montreal.

We have stated previously that preparations of A.P.L. (anterior-pituitary-like hormone) from pregnancy urine or placenta do not lead to maturation of follicles or formation of corpora lutea in the ovary of the hypophysectomized rat,[1, 2] but only to luteinization of theca cells. Smith,[3] however, who repeated this experiment with the commercial preparation of the anterior pituitary-like hormone prepared from pregnancy urine bearing the name Antuitrin S., found that after such treatment new corpora lutea could be seen in the ovaries of both pre- and post-pubertal hypophysectomized rats; these were of the same type as the normal corpora lutea of the postpubertal animal. Thinking that the difference in results might be due to a difference in the preparations used, we repeated our experiment on 10 prepubertal (22-day-old) rats, using Antuitrin S.,* but the results

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[1] Collip, Selye and Thomson, *Nature*, 1933, **131**, 56.

[2] Selye, Collip and Thomson, Proc. Soc. Exp. Biol. and Med., 1933, **30**, 780.

[3] Leonard and Smith, Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1248.

* We used the standard commercial preparation, but Dr. Smith has informed us that the material employed in his experiments was a special sample of greater purity.

were the same as in our previous experiments. The ovaries of these rats showed no corpora lutea, and the only effect of the treatment was thecal luteinization.

It occurred to us that the difference in the results obtained by Smith and by us might be due to differences in the 2 colonies of rats. We decided, therefore, to repeat our experiments on a different animal species. The mouse seemed to be especially suited to an experiment of this type, because the corpora lutea existing at the time of hypophysectomy disappear very rapidly after operation in the non-pregnant animal, and any normal corpora lutea seen in the ovary a few weeks after hypophysectomy would have to be ascribed to the treatment.

Fourteen adult female mice were hypophysectomized for this experiment, and after a period of 2 weeks A.P.L. treatment was initiated in 10 of them; 4 were left as untreated controls. We gave 5 units daily for 9 days, and killed all the animals, including the controls, on the 10th day. We have not seen normal corpora lutea in any of these animals, and the only difference between the treated and the untreated animals was that the theca showed signs of luteal transformation in the former group, while in the latter this was not the case.

These experiments show that in the postpubertal mouse treatment with A.P.L. does not lead to the formation of corpora lutea, but only to luteinization of the theca cells.

7086 C

Heterophile Ophthalmic Allergy. Reactions to Landsteiner Conjugates.*

A. C. KURTZ AND R. R. MADISON. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

It was tentatively concluded from previous data from this laboratory[1] that the heterophile relationships between certain natural antigens are not necessarily the same in local and systemic specific immune reactions. We have extended these comparisons to include

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* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Chambers, J. V., PROC. SOC. EXP. BIOL. AND MED., 1933, 30, 874.

the known common or heterophile factor in certain artificial protein-crystalloid conjugates.

The native proteins selected for this comparison were horse serum (HS), cow serum (CS) and egg-white (EW). These proteins were benzoylated by the technic outlined previously,[2] the quantitative relationships being such that the average protein molecule of the final product (BHS, BCS, BEW) is the "carrier" of approximately 25 benzoyl radicals. Ophthalmic sensitivity was induced by the Seegal technic; unilateral injection into the anterior chamber of the rabbit eye. The subsequent local allergic test was made by intravenous injection, doses, time intervals and anaphylactic criteria being the same as those used by the Seegals.[3]

Preliminary tests showed that the 3 native proteins (HS, CS and EW) are strictly specific in their ophthalmic antigenicity when tested by the Seegal technic, no demonstrable cross-reaction or cross-desensitization being noted in 27 control tests. A series of 36 tests with the benzoylated proteins is recorded in Table I, the recorded data in each case being a composite picture of 4 parallel tests with different rabbits. No suggestion of a benzoyl cross-reaction is apparent from this table. Since the recorded reactions were independent of the order in which the tests were made on the same rabbit, there is no suggestion of a benzoyl cross-desensitization.

TABLE I.
Heterophile relationships of partially Benzoylated native proteins.

Groups of rabbits locally sensitized to:	Unilateral ophthalmic reaction following intravenous injection with: BHS	BCS	BEW
BHS	++++	0	0
BCS	0	++++	0
BEW	0	0	++++

A second series of tests was made of the possible cross-reactions between the native and partially benzoylated protein "carriers". Eyes locally sensitized to BEW, for example, were found to react with equal severity to intravenous injection with BEW or EW, if the tests are made in this order (Table II). Eyes locally sensitized to EW, however, react with full severity to EW; but do not react on subsequent test 24 hours later with BEW. There is, therefore, a distinct "carrier" cross-reaction between the native and benzoylated proteins, the benzoylated "carrier", however, apparently serving as an ineffective desensitizing agent for the native protein.

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[2] Kurtz, A. C., Sox, H. C., and Manwaring, W. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 138.

[3] Seegal, D., and Seegal, B. C., *J. Exp. Med.*, 1931, **54**, 249.

TABLE II.
Cross-reactions with Native and Benzoylated Protein "Carriers".

Group of rabbits sensitized to:	Unilateral ophthalmic allergy following intravenous injection with:		
	BEW	EW	BEW
BEW	++++	++++	—
EW	—	++++	0

The reactions recorded in this paper are all of them strictly unilateral ophthalmic responses. On repeated intravenous tests on the same rabbit, "sympathetic allergy" is eventually elicited in the non-sensitized or control eye. These bilateral responses were originally described by the Seegals[4] and are apparently non-specific in character.

7087 C

Internal Migration of Ova in the Cat.*

J. E. MARKEE AND J. C. HINSEY.

From the Department of Anatomy, Stanford University.

During other investigations, we have had occasion to interrupt one uterine tube to study the changes which might occur during pregnancy in the occupied and unoccupied horns. This report deals with observations on the uterine horns of 7 cats in which the right uterine tube was interrupted in each case a number of weeks before the animal was bred.

In 3 animals, the operative procedure consisted merely of ligation (with silk thread) of the right uterine tube in 2 places and section between the ligatures. These animals were bred and were sacrificed in the 7th week of pregnancy. Fetuses were found in both horns in all 3 animals. We were unable to find any macroscopic evidence of regeneration of the uterine tubes and in 2 animals the silk ligatures were found encircling the cut ends. Further evidence of migration of the ova from the unoperated to the operated side was obtained by a study of serial sections of both fragments of the tube and the tubal end of the uterine horn from the operated side of one of the animals. No histological evidence of a reestablishment of the lumen was present.

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[4] Seegal, B. C., Seegal, D., and Khorazo, D., *J. Immunol.*, 1933, **25**, 207.

* This study was conducted with the aid of a grant from the Sex Division of the National Research Council and of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

In order to test further the possibility of migration from one horn to the other, we transected the uterine tubes in a second series of cats. Here we sectioned the uterine tube midway between its 2 ends, cauterized the free end of the ovarian half and buried the free end of the uterine half in the muscle of the uterine horn. When these animals were sacrificed at different intervals during their pregnancies, there were fetuses in both horns of 3 of the 4 cats. In all of them, what had been the free end of the uterine half of the tube was still buried in the uterine musculature and the silk thread used to sew the tube into the uterine muscle was still in place.

In this series of 7 cats in which the right uterine tube had been sectioned, there were 21 fetuses with a distribution of 10 in the right horns and 11 in the left ones. The average litter was 3. In another series of 13 animals which had been subjected to another abdominal operation, there was an average litter of 4.7. This indicates that there was a reduction in the average number of fetuses in the litters following section of the right uterine tube. It also demonstrates a fairly equal distribution of fetuses in the 2 uterine horns.

There are only 2 ways in which transmigration of ova can occur. In species in which the ovarian bursa is incomplete, migration may occur across the peritoneal cavity, into and down the opposite uterine tube. Since the right uterine tube had been interrupted in these animals, ova could not possibly have gained entrance into the right uterine horn through the tube. Obviously, the ova passed down the tube and horn on the unoperated side to reach the rather long (2 to 3 cm.) common portion of the uterus, and then passed up the horn in the operated side. This brings up the question of how these ova were transported in their passage from one horn to the other. Although Parker[1] has shown that on the endometrium of mammals there is a narrow band of cilia that beat upwards, it does not seem probable that they could have carried the ova up the operated horn. Evidence of a reverse peristalsis during coitus has been presented by Evans[2] in the dog and by Hartman and Ball[3] in the rat. As far as we know, there are no observations which would support the occurrence of such a reverse peristalsis at the time of implantation as would be required to explain our observations. A third possible explanation occurs to us, namely that the uterine horns may contract alternately with sufficient force to drive the uterine contents from one horn to the other. If this were the case, ova might be carried from one horn to the other in those animals which possess a common portion of the uterus of adequate size. This hypothesis would involve the assumption that the cervix and the utero-tubal junctions were effectively closed. Although there is no data at present bearing on this point in the cat, Kelly[4] has shown that the utero-tubal junction is blocked in the guinea pig by a valve-like flap of tissue and Allen[5] mentions a similar fold in the mouse.

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[1] Parker, G. H., *Phil. Trans. Roy. Soc. B.*, 1931, **219**, 381.

[2] Evans, E. I., *Am. J. Physiol.*, 1933, **105**, 287.

[3] Hartman, C. G., and Ball, J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 312.

Internal migration of ova has been studied in a number of different animals. Negative results were obtained in the rat by Doncaster and Marshall,[6] King,[7] Arai,[8] and Rathbone and McGuinness,[9] in the opossum by Hartman,[10] in the guinea pig by Asdell,[11] and Kelly,[12] and in the rabbit by Kinney.[13] Our observations give experimental proof in the cat for those which Corner[14] made on the domestic pig by comparing the number of corpora lutea in each ovary with the number of fetuses in the respective horns. It should be noted that both the cat and the domestic pig have rather a long common portion of the uterus.

The uterine horns of the one cat in which transmigration did not occur seem worthy of especial mention. There were 2 young in the left horn (3.57 and 3.59 gm.) and none in the right. The volume of the left horn (empty) was 12.5 cc., that of the right 6.0 cc. The volumes of the longitudinal muscle, circular muscle, endometrium, connective tissue (between the muscle layers) and the lumen were determined by a method which will be described in detail in a later publication. In the left horn, the longitudinal muscle made up 28% of the volume, the circular muscle 35%, the endometrium 19%, the lumen 6%, and the connective tissue 11%. In the right unoccupied horn, the longitudinal muscle made up 32% of the volume, the circular muscle 32%, the endometrium 16%, the lumen 2%, and the connective tissue 18%. It is self-evident that there is a local factor that influences the growth of the uterine tissues during pregnancy in the cat. This local factor is of considerable consequence since it causes the occupied horn to grow to dimensions double that of the unoccupied one which is presumably subjected to the same hormonal changes. This growth is accomplished by an approximate relative increase in each of the component tissues of the uterine horns as is shown by the fact that the percentage content of the longitudinal and circular muscle layers, endometrium and connective tissue (between muscle layers) on the 2 sides shows only slight variations which are probably within the error of our method. The growth of the muscle at the implantation sites might be attributed to a work hypertrophy but this cannot explain the greater growth in the portions between the implantation sites of the left horn. This latter is easily seen by a comparison of microscopic cross sections of the right and left horns (between implantation sites) where the latter is considerably larger. This difference was further shown in our direct measurements. This local factor is being studied in greater detail in the rabbit.

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[4] Kelly, G. L., *Am. J. Anat.*, 1927, **40**, 373.

[5] Allen, E., *Am. J. Anat.*, 1922, **30**, 297.

[6] Doncaster, L., and Marshall, F. H. A., *J. Genetics*, 1910, **1**, 70.

[7] King, H. D., *J. Exp. Zool.*, 1911, **10**, 381.

[8] Arai, H., *Am. J. Anat.*, 1921, **28**, 59.

[9] Rathbone, R. H., and McGuinness, J. E., 1927, unpublished work from the Department of Anatomy, Stanford University.

[10] Hartman, C. G., *Am. J. Anat.*, 1923, **32**, 353.

[11] Asdell, S., *Brit. J. Exp. Biol.*, 1923, **1**, 473.

[12] Kelly, G. L., *Anat. Rec.*, 1928, **40**, 365.

[13] Kinney, P. B., *Am. J. Obst. and Gynec.*, 1924, **8**, 198.

[14] Corner, G. W., *Johns Hopkins Hosp. Bull.*, 1921, **32**, 78.

7088 C

Pregnancy Following Bilateral Section of the Cervical Sympathetic Trunks in the Rabbit.*

J. C. HINSEY AND J. E. MARKEE.

From the Department of Anatomy, Stanford University.

The ovulation which follows coitus is known to depend upon the activity of the anterior lobe of the hypophysis. This activity must depend upon the central nervous system. However, at the present time the efferent pathway to the anterior lobe of the hypophysis is only a matter of conjecture as to whether it is direct or indirect. The tractus supraoptico-hypophyseus passes from the nucleus supraopticus to the posterior lobe (Greving[1]). The only innervation which is known for the anterior lobe is from the carotid plexus. If the pathway from the central nervous system to the anterior lobe

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* This investigation was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University and a grant from the Sex Division of the National Research Council.

[1] Greving, R., 1931, p. 188, in L. R. Müller's *Lebensnerven und Lebenstriebe,* 3rd Edition. Julius Springer, Berlin.

of the hypophysis is a direct one, it is conceivable that it may pass to it by way of the carotid plexus. Cannon and his coworkers[2] showed in one cat that conception and delivery could take place following complete removal of the thoracolumbar sympathetic outflow from the central nervous system.

In a series of 8 adult rabbits which had been isolated for 3 weeks, the cervical sympathetic trunk was sectioned bilaterally below the superior cervical sympathetic ganglion. In one of the animals, the superior cervical sympathetic ganglion was removed bilaterally. The animals were bred about one week after the operation. Each of them became pregnant and delivered an average litter of 7 plus young. Autopsies were done to control the operations. The observations show that the pathway through the superior cervical sympathetic ganglion is not essential for ovulation in the rabbit. While the possibility must be recognized that there may be some slight contribution to the carotid plexus from the other cervical sympathetic ganglia, by far the greatest portion of this plexus is derived from the superior ganglion.

When this evidence in the rabbit is combined with that of Cannon in the cat, it seems that there are only 2 other possibilities for efferent pathways to the anterior lobe of the hypophysis: either there are contributions to the carotid plexus from cranial nerves such as Cobb and Finesinger[3] and Chorobski and Penfield[4] have described from the greater superficial petrosal nerve or the pathways from the hypothalamus must activate the posterior lobe of the hypophysis which in turn may exert an influence on the anterior lobe by hormonal transmission.

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[2] Cannon, W. B., Newton, H. F., Bright, E. M., Menkin, V., and Moore, R. M., *Am. J. Physiol.*, 1929, **89**, 84.

[3] Cobb, S., and Finesinger, J. E., *Arch. Neurol. and Psychiat.*, 1932, **28**, 1243.

[4] Chorobski, J., and Penfield, W., *Arch. Neurol. and Psychiat.*, 1932, **28**, 1257.

7089 C

Effect of Chaulmoogric Acid Derivatives on Lipolytic Activity in vitro.*

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Following the demonstration by Shaw-Mackenzie[1] that sodium gynocardate specifically activates pancreatic lipase *in vitro* by some 100% under the experimental conditions employed, Rogers[2] advanced the hypothesis that the mode of action of chaulmoogrates in leprosy therapy consists of a similar activation *in vivo* which would facilitate humoral destruction of the fatty capsule of *Mycobacterium leprae.* Significant differences were found by Rogers in the ethyl-butyrase activity of sera, shipped on ice from India, of treated and untreated lepers, the former approximating normal. Neill and Dewar[3] have been unable, however, to confirm these observations in a more extensive study of fresh leprous sera.

We have previously studied[4] by Loevenhart's method[5] the *in vivo* effects of certain antileprotic drugs on the ethyl-butyrase activity of tissues of rats experimentally infected with a standard strain of rat leprosy supplied by Dr. E. L. Walker. No reversion of the lipolytic activity to normal was brought about by any of the chaulmoogrates studied. Preliminary *in vitro* ethyl-butyrase studies on liver bries, in which 1 cc. of a 1% solution of the test agent was added to the Loevenhart reaction mixture, revealed an inhibition rather than an activation, varying to some extent with the type of chaulmoograte used. For the following drugs† averages of 5 determinations expressed as per cent of original activity lost, were: sodium gynocardate, 60%; alepol, 40%; sodium chaulmoogryl-p-phenetidine sulfonate, 90%; sodium dihydrochaulmoogryl-p-phenetidine sulfonate, 90%; sodium chaulmoogryl glycinate, 65%; hydroxymercuriethoxy chaulmoogric anhydride[6] (saturated), 10%; and sodium dichaulmoogryl-β-glycerophosphate, 15%.

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* Part of a cooperative study of the chemotherapy of leprosy conducted by the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund for Medical Research.

[1] Shaw-Mackenzie, J. A., *Med. Press and Circ.*, 1920, **2**, 122; *J. Trop. Med. and Hyg.*, 1921, **24**, 161.

[2] Rogers, L., *Brit. Med. J.*, 1923, **2**, 11; *Lancet,* 1924, **2**, 1297.

[3] Neill, M. H., and Dewar, M. M., *Pub. Health Bull.*, 1927, **168**, 1.

[4] Emerson, G., Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 150; 1933, **31**, 18.

[5] Kastle, J. H., and Loevenhart, A. S., *Am. Chem. J.*, 1900, **24**, 491; Loevenhart, A. S., *Am. J. Physiol.*, 1902, **6**, 331.

† All but the last drug were prepared and generously supplied to us by Dr. Richard Wrenshall, Department of Chemistry, University of Hawaii.

Addition of an only slightly soluble salt of a weak acid (sodium gynocardate) to an enzyme-substrate system results in considerable inaccuracy in the determination of the true end-point on titration unless careful comparison with a color standard is made, but the magnitude of the activating effect observed by Shaw-Mackenzie indicated a more significant factor was involved. It occurred to us that since in Shaw-Mackenzie's experiments sodium gynocardate was added to a glycerol pancreatic extract and the hydrolysis of the olive oil substrate allowed to proceed for 18 to 24 hours before titration, considerable enzymatic synthesis of chaulmoogryl glycerides must also take place in this time, resulting in the removal of weaker acids (chaulmoogric and hydnocarpic) with replacement by stronger acids from the glycerides of the olive oil substrate, thus leading to an apparent higher acid value on titration. We have, therefore, repeated Shaw-Mackenzie's experiments using both an aqueous and a 60% glycerol extract of fresh dog pancreas. Inhibition was found in the case of the aqueous extract while only slight apparent activation was observed with the glycerol extract.

Summary. In addition to the previously demonstrated lack of *in vivo* lipase activation by the antileprotic chaulmoogrates, it is shown that the apparent *in vitro* activation is not real, but is more likely an artefact due to the disturbance of equilibria brought about by the introduction of a salt of a weak acid into an enzyme reaction system containing large amounts of free glycerol. If the chaulmoogrates exert any indirect action in leprosy therapy besides their direct bactericidal action, it is improbable that the fat-splitting ferment is involved.

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[6] Dean, A. L., Wrenshall, R., and Fujimoto, G., *J. Am. Chem. Soc.*, 1925, **47**, 403.

7090 C

Comparative Biological Activity of Seven New Water-soluble Chaulmoogric Acid Derivatives.*

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Rogers[1] introduced the first water-soluble chaulmoogra derivative intended for intravenous injection in leprosy, which was recognized to be a systemic disease most rationally treated by administering drugs for systemic effect. Due to the hemolytic, local irritative, sclerosing and other undesirable properties of sodium gynocardate, its intravenous use in humans was generally abandoned even though promising results had been obtained in a considerable number of lepers. No other soluble chaulmoograte has been developed sufficiently free from the disadvantages of "Alepol" (sodium hydnocarpate) or sodium gynocardate to warrant its continued intravenous use. This report deals with several compounds which appear from various theoretical considerations to be preferable to the chaulmoogra soaps.

Seven new water-soluble derivatives of chaulmoogric acid† have been examined pharmacologically and 3 of these have been tested further by a standardized method for antileprotic efficacy as measured by their influence on the course of an experimental leprous infection in rats. The strain of rats, infection, the time and route of administering the drugs, the diet, size of treated and untreated groups, and all other controllable experimental conditions were identical with those used in work previously described.[2] Further experimental treatment is being continued for a more extended period with the more promising agents.

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* Part of a cooperative study of the chemotherapy of leprosy conducted by the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund for Medical Research.

[1] Rogers, L., *Lancet*, 1916, **1**, 288; *Brit. Med. J.*, 1916, **2**, 550.

† Those compounds designated by numbers are preparations synthesized and generously supplied by Dr. Richard Wrenshall, of the Chemistry Department of the University of Hawaii; those designated by a letter were synthesized by one of us (G. A. E.) from chaulmoogric acid obtained from redistilled pure ethyl chaulmoograte supplied by Dr. Wrenshall.

[2] Anderson, H. H., Emerson, G. A., and Leake, C. D., *Internat. J. Leprosy*, in press.

TABLE I.

Biological Activity of Water Soluble Chaulmoogric Acid Derivatives.

Drug % of drug in water	Toxicity, acute subcutaneous in rats Gm./Kg.	Toxicity, acute intravenous in rats Gm./Kg.	Total tolerated dose* in treated leprous rats in 6 mo. Gm./Kg.	No. deaths during treatment	Aver. wt. variation in treated leprous rats	Aver. size of leproma at end of treatment Sq.cm.	Total no. of sloughs occurring during treatment	Bactericidal concentration in vitro†	Minimum hemolytic concentration on human blood in vitro	Local effects noted of acute toxicity in surviving rats
No. 104 3% "Alepol" (Na hydnocarpate)	2.0 7/7 died in 24 hr.	0.1-0.125 3/5 died in 2 min.	0.445	3/12	+8	7.4	22	1:20,000	1:20,000 in 3 hrs.	Necrosis in 9/18 rats
Drug "A" 3% (Na dichaulmoogryl-β-glycerophosphate)	2.0 3/5 died in 6 to 24 hr.	1.25 3/6 died in 2 min. to 30 hr.	2.475	0/8	+65	4.2	4	1:20,000	1:500 in 20 hr.	No necrosis at injection site when given subcutaneously
Drug "B" 5% (Na chaulmoogryl glutamate)	2.0 3/5 died in 24 to 48 hr.	0.15-0.25 3/6 died in 10 min. to 24 hrs.	not tested	not tested	—	—	—	1:1,000	1:40,000 in 3 1/2 hr.	No necrosis at injection site when given subcutaneously
No. 1141 3% (Na chaulmoogryl glycinate)	1.0 4/5 died in 24 to 48 hr.	0.15-0.20 3/4 died in 6 min. to 10 min.	0.420	3/8	+56	5.4	5	1:1,000	1:10,000 in 30 min.	Necrosis in 4/12 rats
No. 661-K 3% (K-iodo-dihydrochaulmoograte)	0.25 4/5 died in 10 to 24 hr.	—	0.410	2/8	+52	9.8	6	1:20,000	1:10,000 in 60 min.	No necrotic effect
No. 2001 5% (Na diethylethanolammonium chaulmoograte)	1.5 3/5 died in 3 to 24 hr.	—	not tested	not tested	—		—	1:10,000	1:20,000 in 45 min.	Necrosis in 7/9 rats
No. 1601 3% (Na chaulmoogryl o-aminobenzoate)	0.5 4/8 died in 24 hr.	—	" "	not tested	—	—	—	1:1,000	1:100,000 in 15 min.	Necrosis in 8/8 rats
No. 2211 5% (choline chaulmoograte)	0.5 5/5 died in 5 min.	—	" "	not tested	—	—	—	1:1,000	1:100,000 in 2 hr.	Necrosis in 1/8 rats

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* Infiltrated lesion site with drug; dose expressed in chaulmoogric acid equivalent. Rats were infected with a standard strain of *Mycobacterium lepra muris* obtained from Drs. Walker and Sweeney.

† *Mycobacterium lepra hominis*, Mary Puhulahula strain (isolated by Dr. E. L. Walker) was grown on Long's liquid media (*Amer. Rev. Tuberc.*, 1926, **13**, 393).

In Table I may be found the tolerated and minimum lethal doses on subcutaneous and intravenous injections in rats, the *in vitro* bactericidal concentration and the effects in experimental rat leprosy of K-iodo-dihydrochaulmoograte (No. 661-K), Na-chaulmoogryl-glycinate (No. 1141) and Na-dichaulmoogryl-β-glycerophosphate (Drug "A")[3] in comparison with those of "Alepol" (No. 104). The average variations in body weight, size of leprous lesion and incidence of sloughing lesions in each group of infected rats during a 6-months treatment period are included. The toxicity and *in vitro* bactericidal activity of Na-chaulmoogryl-o-aminobenzoate (No. 1601),[4] diethyl-ethanolammonium chaulmoograte (No. 2001), choline chaulmoograte (No. 2211) and Na-chaulmoogryl glutamate (Drug "B") are also shown.

Among infected but untreated control groups observed in earlier studies the mortality from the disease, pneumonia and other causes was 33% in a year, while here 3 of 8 infected control animals have died in 7 months. The mortality ratios of similar leprosy infected rats under treatment with the various drugs are shown in Table I. Average calculated doses of chaulmoogric acid per drug for each of the 3 treatment periods per group of treated rats were: "Alepol," 200, 125 and 120 mg./kg.; K-iodo-dihydrochaulmoograte, 75, 135 and 200 mg./kg.; Na-chaulmoogryl-glycinate, 70, 125 and 225 mg./kg.; and Na-dichaulmoogryl-β-glycerophosphate, 625, 1150 and 700 mg./kg. The apparent superiority of the latter compound in comparison with the others may be due to the fact that we were able to give it in larger amounts, especially with reference to chaulmoogric acid content, without toxic or local effects. This drug is probably hydrolyzed more slowly than the others in the body, liberating a relatively non-toxic solubilizing group and prolonging the presence of the presumably effective chaulmoogra remainder. The modifying factor of availability of the chaulmoogric acid must be considered, however, since both drugs 1141 and "A" rank with the ethyl esters in therapeutic effectiveness although given in much smaller amounts. Our findings in this study with new water-soluble chaulmoogratess support our previous conclusions.[2] The importance of unsaturation and optical activity of chaulmoogra derivatives in probable therapeutic value is emphasized by the ineffectiveness of the saturated HI substituted soap, drug 661-K.

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[3] Emerson, G. A., in press.

[4] Santiago, S., and West, A. P., *Philipp. J. Sci.*, 1927, **33**, 265; independently prepared by Dr. Wrenshall, through a synthesis giving greatly improved yields (personal communication, 1932).

Since these agents were studied from the standpoint of their possible intravenous use it was felt advisable to test their hemolytic activity on human blood *in vitro*. Dikshit[5] has shown that the degree of hemolysis produced by "Alepol" and other chaulmoogra soaps is the same as that brought about by the Na oleate of ordinary washing soaps. This action is modified by the formation of insoluble Ca salts, if the drugs are dissolved in Locke's solution or if serum is added to Locke's solution as Dikshit found.[5] We compared the hemolytic properties of the 8 derivatives using concentrations from 1:500 to 1:100,000 in 0.9% NaCl solution. One-tenth of a cc. of washed human red cells was added to 10 cc. of the drug solution and observations were made over a 48-hour period. Na stearate, used as control, hemolyzed blood cells at the same concentrations as "Alepol". No hemolysis occurred in any of the Drug "A" solutions within the first 12 hours. Other results are indicated in Table I.

Studies on the metabolism of chaulmoogryl derivatives which contain a physiologically inert or harmless solubilizing radicle rather than a toxic one are in progress.

Summary. On the basis of *in vitro* bactericidal and hemolytic action, intravenous toxicity, tolerance, and effectiveness in experimental rat leprosy, Na-dichaulmoogryl-β-glycerophosphate ("chaulphosphate" for convenience), appears superior for possible use in the intravenous treatment of leprosy than other representative water-soluble chaulmoogra derivatives, such as Na-hydnocarpate ("Alepol"), K-iodo-dihydrochaulmoograte, Na-chaulmoogryl-glycinate, Na-chaulmoogryl-o-aminobenzoate, diethyl-ethanolammonium chaulmoograte, and choline chaulmoograte.

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[5] Dikshit, B. B., *Ind. J. Med. Res.*, 1932, **19**, 775.

7091 C

The Laxative Principle in Prunes.

GEORGE A. EMERSON.* (Introduced by C. D. Leake.)

From the Pharmacological Laboratory of the University of California Medical School, San Francisco.

No satisfactory study of the widely recognized cathartic effect of the French or Santa Clara variety of prune has ever been reported as far as we know. The purgative action appears in foodstuffs derived from prunes as well as in the dried prune itself. The apparent thermostability of the evacuant principle may be the factor leading Sollmann[1] and others to include prunes with the colloid and emollient laxatives which act "mainly by retaining water in the intestine by imbibition, thus modifying the bulk and consistency of the feces, so that these are more easily expelled." Water-soluble substances in prunes have previously been held to play only a subsidiary rôle in catharsis; Sollmann[1] explains their action thus: "in fruits (prunes, ... , etc.) this colloid action is supported by the organic acids and sugars."

We studied the cathartic efficacy of the soluble compounds present in dried prunes in an attempt to isolate the active substance responsible. Since such an active principle is present in very high dilution, routine chemical tests yield little information. The color reactions described by Tumin Katti and Beal, Fuller, Warren, and others[2] for anthraquinone derivatives which act through an irritant effect, are negative according to Mrak and Smith[3] for all prune extracts tested, indicating the absence of emodin-like compounds. The $FeCl_3$ test for phenolic groups is positive.

Three methods were used to evaluate the potency of various extracts. In spite of Sollmann's criticism[1] of the Magnus technique[4] with isolated gut, it is a convenient one to determine whether or not activity is present in materials tested. Preliminary tests were made by this technique on various prune preparations and the results so obtained were in accord with those found in intact animals by either Macht's[5] or Eddy's[6] method.

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* This effort was conducted in cooperation with W. V. Cruess, E. M. Mrak, and Clifford Smith of the Division of Viticulture and Fruit Products of the Department of Agriculture of the University of California, Berkeley, who prepared most of the extracts studied.

[1] Sollmann, T., *Manual of Pharmacology,* Philadelphia, 4th Ed., 1932, 244, 228.

[2] Warren, L. E., *Am. J. Pharm.,* 1914, **44**, 86; Beal and Tumin Katti, *J. Am. Pharm. Assn.,* 1925, **14**, 865; Tumin Katti and Beal, *J. Am. Pharm. Assn.,* 1926, **15**, 847; Fuller, H. C., *J. Assn. Off. Ag. Chem.,* 1926, **9**, 306; 1927, **10**, 364.

[3] Personal communication, 1933, confirming G. A. Pitman.

[4] Magnus, R., *Arch. ges. Physiol.,* 1904, **102**, 123.

Actions of prune extracts on isolated rabbit, guinea pig, dog and cat jejunum or duodenum in either Sollmann and Rademaekers'[7] or Sollmann and von Oettingen's[8] solutions are summarized in Table I. The individual responses were confirmed at least in triplicate, and the tabulated results represent some 200 separate experiments. The water extracts were prepared according to the directions of Mrak and Cruess.[9] All other extracts were thoroughly freed of solvent by prolonged evaporation *in vacuo* and dissolved or suspended in water before being added to the bath. None of the extracts was sufficiently acidic or basic to influence the observed effect.

From the results in Table I it would appear that there are 2, and perhaps 3, different effects demonstrable. The first, which increases both tonus and amplitude of contraction, is constantly shown by an agent soluble in water, alcohol and pyridine, but which cannot be completely removed from finely ground and well desiccated prune pulp even on exhaustive extraction. This prune constituent loses its activity on extensive hydrolysis. The other effects noted with different extracts may be due to its split products which may have different solubilities, and which themselves may be present in whole prunes. A marked diminution of tonus and complete suppression of peristalsis is noted with acetone extracts. The agent involved here may be responsible for the primary inhibition of tonus and peristalsis which constantly occurs before the isolated gut shows the remarkable increase in activity on adding alcoholic or aqueous extracts to the bath. A third effect, as yet unexplained, occurs with basic lead acetate precipitates, which reduce tonus while causing an increase in amplitude of contraction. This effect is under further investigation.

Comparisons of kymograph tracings obtained by the Magnus technique from water extracts of prunes with those obtained from 16 other representative cathartics of various chemical types, show a resemblance only to diacetyl dioxyphenyl isatin ("Isacen"). Di-

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[5] Macht, D. I., and Barba-Gose, *J. Am. Pharm. Assn.*, 1931, **20**, 558; Macht, D. I., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1272.

[6] Eddy, N. B., *J. Pharmacol. Exp. Therap.*, 1932, **45**, 339.

[7] Sollmann, T., and Rademaekers, A., *Arch. Intern. Pharmacol.*, 1925, **31**, 39.

[8] Sollmann, T., von Oettingen, W. F., and Ishikawa, Y., *Am. J. Physiol.*, 1928, **85**, 118; Whitehead, R. W., *Am. J. Physiol.*, 1929, **89**, 253.

[9] Mrak, E. M., and Cruess, W. V., *Univ. Calif. Ag. Exp. Sta. Bull.*, 1929, **483**, 1.

TABLE I.
Effect of Prune Extracts on isolated rabbit Jejunum or Duodenum.

				Effects on	
Source	Extract	Treatment	Dilution†	Tonus	Amplitude
Prune Pulp	aqueous	none	1:5,000	++	++
		charcoal	1:100	0	0
		filter cell	1:5,000	++	++
		kaolin	1:1,000	+	+
		acid hydrolysis	1:100	0	0
		basic "	1:100	0	0
		yeast fermentation	1:1,000	0	+
		Pb acetate	1:100	0	0
		heating (autoclave)	1:1,000	++	++
	alcoholic	none	1:5,000	++	++
		charcoal	1:100	0	0
		filter cell	1:1,000	++	++
		Pb acetate	1:100	0	0
	acetone	none	1:1,000	—	—
		acid hydrolysis	1:500	0	—
		emulsin "	1:500	—	—
		maltose "	1:500	—	—
	ether	none	1:100*	0	0
	pyridine	"	1:500	++	++
		exhaustive extraction			
	—	ether	1:1,000	+	++
	—	alcohol	1:1,000	+	+
	—	water	1:1,000	++	++
Charcoal Adsorbate	—	elution at pH 9.0	1:100	0	0
of aqueous or	—	acid elution	1:100	0	0
alcoholic extracts	—	alcoholic "	1:100	0	0
	—	acetone "	1:500	—	—
Lead acetate ppt.	—	H_2S to remove Pb	1:500	—	—
of aqueous or	—	same, of first fraction			
alcoholic extracts		only	1:100	+	+
Alcoholic Extract	ether	none	1:500*	0	±
	acetone	"	1:500	—	—
Prune Pits	aqueous	"	1:100	+	+

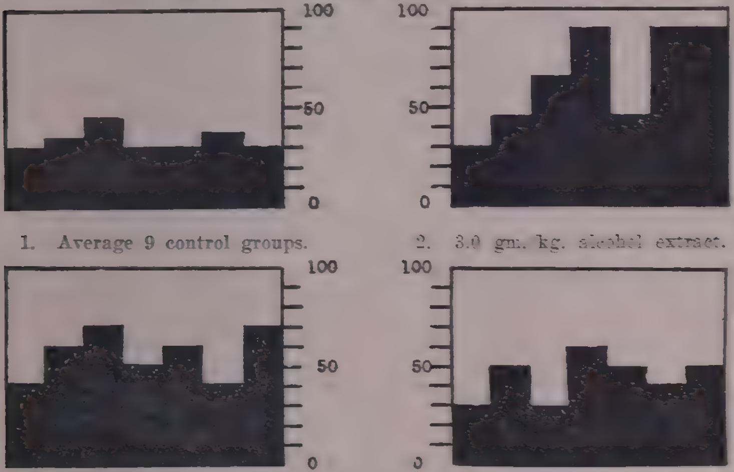
† = total prune solids / volume of bath
* = suspension

++ = marked increase
+ = definite, but smaller increase
0 = no effect
— = marked decrease

hydroxyphenyl isatin was prepared and gives the same type of physiological response with isolated gut; it also reacts with $FeCl_3$, while diacetyl di-oxyphenyl isatin does not. Chlorogenic and caffeic acids also cause an immediate increase in tonus and amplitude of contraction similar to that caused by prune extracts. These acids were tested because of the similarity in color reaction with that developed in the prune phenolic compound on treatment with $FeCl_3$.

These results on the isolated gut were confirmed by experiments using Macht's method,[7] but since the observed mean deviation was high and a minimum of 10 rats had to be sacrificed for each determination, we made use of this technique for only a selected group of prune preparations. Results expressed as the average per cent of the total length of intestine, from pylorus to anus, traversed in 50 minutes by a test meal of a suspension of wood charcoal in gum tragacanth incorporated with the extract to be tested, in groups of 10 rats, were: controls, 55 ± 6%; water extract of prunes clarified with a filter cell, 1.0 gm./kg., 63 ± 5%; pulp of the whole dried prune, 1.0 gm./kg., 66 ± 10%; and the alcoholic extract of prunes, 1.0 gm./kg., 69 ± 8%.

Average Effects of Prunes on Defecation in Groups of 10 Rabbits by Eddy's Method.
Abscissae: time in hours. Ordinates: percentage of animals defecating.



1. Average 9 control groups.
2. 3.0 gm./kg. alcohol extract.
3. 0.5 gm./kg. water extract.
4. 0.5 gm./kg. dried prune pulp.

Eddy's technique[5] was more satisfactory. Representative observations are shown in the charts of Fig. 1. The results obtained on isolated gut were again confirmed. The discrepancy between Eddy's control group figures and those in Fig. 1 is probably due to the more highly constipating diet deliberately used in our experiments.

Summary. As tested by the techniques of Magnus, Macht, and Eddy, the laxative properties of prunes are not due entirely to colloidal or emollient effects in the intestine, as has been previously supposed, but would seem to depend also on the presence of an agent soluble in water, alcohol and pyridine. This active principle in prunes which is broken down by strong hydrolysis has otherwise certain chemical and physiological properties similar to di-hydroxyphenyl isatin and to caffeic and chlorogenic acid.

7092 P

Absolute Values and Temperature Coefficients of Arbacia Egg Respiration.*

B. B. RUBENSTEIN AND R. W. GERARD.

From the Department of Physiology, University of Chicago.

Though all workers have agreed that the respiration of Arbacia eggs increases about 5 times on fertilization, there is disagreement as to the absolute values. Since some theoretical importance attaches to these magnitudes, it seemed desirable to resolve the discrepancies. The most recent determinations by Tang[1] and Tang and Gerard[2] and by Whitaker[3] all utilized the Warburg manometric technique, yet apparently differed by a factor of nearly 2. Expressed as cubic millimeters of oxygen used per 10 cu. mm. of eggs per hour (at 21°C.), the former observers obtained for resting eggs, 0.8; the latter, 0.4-0.5. Differences in technique included: securing the eggs and preparing the suspension; shaking in the manometers; determination of egg volume; and experimental temperature. All these points have been checked by direct comparison of the 2 techniques at each step. The figure obtained in the present series is 0.9.

The results show that whether eggs are obtained by picking out ovaries or allowing the eggs to shed from the gonopores of the half shell is immaterial. Likewise, mild hand centrifuging or filtering through cheese cloth has no effect. Further, both the shaking rates used permitted an adequate oxygen supply without injury to the eggs.

A major difference appeared, however, in the techniques for evaluating egg volume. The egg volume determined by centrifuging a given amount of suspension to constant egg mass was regularly about 80% greater than that calculated from the egg diameter (determined with ocular micrometer) and cell count (with haemocytometer). That the centrifuge method is in error, due to imperfect packing, egg jelly, etc., was further shown by examining an egg mass in the Harvey centrifuge-microscope. During strong centrifuging individual eggs remained widely separated, often by a full egg diameter. Applying this correction to Whitaker's figures, they are in essential agreement with ours and Tang's.

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* Aided in part by a grant from the Rockefeller Foundation to the University of Chicago.

[1] Tang, P. S., *Biol. Bull.*, 1931, **60**, 242.

[2] Tang, P. S., and Gerard, R. W., *J. Cell. Comp. Physiol.*, 1932, **1**, 503.

[3] Whitaker, D. M., *J. Gen. Physiol.*, 1933, **16**, 475, 497.

Of especial interest was the influence of temperature on egg respiration. The temperature coefficient for fertilized eggs was slightly under 2, between 11° and 30°C., essentially as reported by Loeb and Wasteneys.[4] The unfertilized eggs, however, show a high coefficient, over 4; so that a 5-fold increase in respiratory rate is true only for intermediate temperatures. At 10° the increase is 10 times and at 31-32°, by slight extrapolation (it is not practical to use these temperatures because of injury), there is no change at all on fertilization. These striking differences in resting and fertilized respiration indicate some fundamental change in the chemistry of the egg. The general reaction: substrate is oxidized, still goes on. Whether the change indicates a shift of master reaction from one member of the concatenated reactions of oxidation to another, or whether it indicates that fertilization introduces an excess of catalyst so that some physical process now becomes the limiting factor in determining oxidation rate, cannot be decided from these experiments.

We wish to acknowledge the kindness of Dr. A. J. Goldforb in permitting us to compare our results with his unpublished ones, which agree on a number of the above points.

7093 P

Ovarian Response of Hypophysectomized Rats to Urinary Follicle-Stimulating Principle.*

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From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Zondek[1] and Kurzrok,[2] by injections in normal immature female rodents, have demonstrated that certain urines (after ovariectomy and the menopause) may induce predominantly a follicular growth. Leonard[3] has shown that the principle in this urine can be differentiated from that in P.U. by the rabbit ovulation test. The difference in the ovarian response elicited by the F.-S.U.‡ extract and the principle in P.U. is a quantitative one in normal animals for P.U. injections also give follicular growth in them. We have found a definite qualitative difference in the effect given by these 2 types of urine in hypophysectomized rats.

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[4] Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1911, **36**, 345.

* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

† National Research Fellow.

[1] Zondek, B., *Klin. Wochenschr.*, 1930, **9**, 393.

[2] Kurzrok, R., 1933, in press.

[3] Leonard, S. L., *Am. J. Physiol.*, 1931, **98**, 406.

We have secured pronounced follicular growth in each of the 12 hypophysectomized rats injected with F.-S.U. extract, treatment having been begun 5-15 days after pituitary ablation.§ This follicular growth takes place within a 5-day period. Even when treatment was continued for 10 days, luteinization occurred only in a small part of the wall of an occasional follicle. The ovaries are very similar to those of normal immature rats whose gonads have been precociously matured by A.P. implants. The effect of the injections is thus opposite to treatments with P.U. extract which does not induce follicular growth after hypophysectomy but causes interstitial hypertrophy and luteinization.

The ovarian weights after treatment with F.-S.U. extract are in the normal range. Structure rather than weight is the better index of a successful replacement therapy.

The injection of F.-S.U. extract has not induced ovulation after hypophysectomy. It brings the follicles to a condition similar to that obtaining in rabbits in heat. Ovulation was secured in 2 pituitaryless rats, however. Each was unilaterally ovariectomized after a 5-day period of F.-S.U. injections and then 100 R.U. of Antuitrin S was given intraperitoneally. The remaining tube of one animal contained 21 ova, that of the other animal 6 ova. All showed polar spindles. In 4 hypophysectomized rats given the same treatment, but after a shorter post-operative period, ovulation did not occur. The ova lay free, however, in the follicular cavity and had spindles.

Concurrent injections of F.-S.U. and P.U. extracts give "augmentation" in normal and hypophysectomized rats. The ovarian weights are greatly in excess of those which would be predicted from adding the increases produced by each of the extracts injected individually. The principle in the F.-S.U. extract appears to be of pituitary origin. It almost completely fulfills the requirements postulated for a follicle-stimulating hormone of the anterior hypophysis.

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‡ Since pregnancy urine is commonly designated as P.U., we have abbreviated the follicle-stimulating urine as F.-S.U., thus avoiding the use of the term "Prolan", which designates a patented product.

§ The urine used in these experiments was from a woman who had passed the menopause and who suffered from migrane. It was secured through the courtesy of Dr. Kurzrok. A urine equivalent of 10 cc. or 20 cc. was injected subcutaneously, each day.

7094 P

Isolation of Streptococci in a Study of the Epidemic of Encephalitis in St. Louis.

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In a previous report[1] the consistent demonstration of streptococci in the spinal fluid obtained from patients during the active stage of encephalitis; the isolation of a streptococcus having peculiar cataphoretic velocity and virulence from the nasopharynx, spinal fluid, and brain; the specific agglutinability of the streptococcus by the encephalitis antistreptococcus serum, and by the serum of convalescent patients; and the production in rabbits of the more important acute clinical and pathological manifestations of the disease, have been recorded. Since then the streptococcus has been isolated from the blood of 5 of 7 patients during the active stage of the disease, it has been found in the nasopharynges and spinal fluids in additional cases, and a large proportion of contacts and non-contacts in the epidemic zone have been found to be carriers of this streptococcus. Thus, the streptococci of 52 (81%) of the different strains isolated from 64 persons in the active stage of the disease (2 to 10 days) had mainly neurotropic cataphoretic velocity. The streptococci of 78 (52%) of the different cultures from 149 well persons and patients with diseases other than encephalitis, in the epidemic zone, had a similar neurotropic cataphoretic velocity. The results in both of these groups were in sharp contrast to the results in 149 well persons and patients with diseases other than encephalitis, remote from the epidemic, in which the streptococci from only 39 (26%) had mainly neurotropic cataphoretic velocity.

Streptococci or diplococci have been demonstrated in or adjacent to the lesions of the brain, and in the sediment of spinal fluids of rabbits and monkeys that developed encephalitis following inoculation with the streptococcus, and with emulsions of the glycerolated brain in fatal cases, and in or adjacent to the lesions in the brains in several fatal cases.

The precipitin reaction with the encephalitis antistreptococcus serum and cleared nasopharyngeal washings, was positive in 78% of 69 active cases, and in 49% of 149 contact and non-contact controls residing in the epidemic zone, as compared with 14% of 159 well persons tested remote from the epidemic.

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[1] Rosenow, E. C., *Proc. of Staff Meetings of The Mayo Clinic*, 1933, **8**, 559.

All (100%) of 6 cases of encephalitis in from the second to the fourth day of the disease gave a strongly positive skin test following injection of the heat-killed streptococcus isolated from the spinal fluid of acute cases. Only 4 (11%) of 37 cases in from the eighth day to the fourteenth day of the disease gave a positive reaction. The incidence of a positive skin test in different groups of persons residing within, and remote from, the epidemic zone ranged from 48 to 72%. The marked reduction in incidence of positive reactions during recovery from encephalitis occurred only in the case of the antigen prepared from the streptococcus of encephalitis. The incidence of positive Schick and Dick tests was about the same among the patients with encephalitis, irrespective of the time of the attack, as it was among well persons, and in all instances reaction was nearly always absent to the antigen prepared with streptococci isolated from the nasopharynges of well persons.

Thus far 4 of 12 strains of streptococci that had manifested characteristic virulence, when placed in milk, resisted heating to 63°C. (145°F.) for 30 minutes and retained their virulence if cultures for viability and animal inoculation were made in tall tubes of dextrose-brain broth.

7095 C

Ether Hyperglycemia.

R. A. PHILLIPS* AND N. E. FREEMAN.* (Introduced by W. B. Cannon.)

From the Laboratories of Physiology, Harvard Medical School.

Glycosuria following etherization was first observed by Reynoso,[1] while the increase of blood sugar accompanying ether anesthesia was first reported by Seelig.[2] Section of the nerves to the liver does not prevent the hyperglycemia resulting from the administration of ether.[3] If the adrenals are inactivated, however, this rise in blood sugar is in large part prevented, as Keeton and Ross[4] showed.

That adrenin rather than the action of sympathetic impulses is the potent factor in liberating sugar from the liver has been shown by Bulatao and Cannon[5] and by Britton.[6] Reid[7] had advanced evidence that the hyperglycemia resulting from etherization is due to secretion of adrenin. But after removal of the adrenal factor ether still produces an increase of blood sugar. The demonstration by Cannon and Bacq,[8] that stimulation of the sympathetic nerves produces sympathin and that sympathin resembles adrenin in action, suggested that the residual hyperglycemia from etherization after adrenal influence had been excluded might result from the action of sympathin. If this idea were correct the level of blood sugar should not rise when ether is administered to completely sympathectomized animals.

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* Medical Fellow of the National Research Council.

Healthy adult cats were maintained in good condition on an adequate diet and fasted for 24 hours before the experiment. Blood (0.1 cc.) was drawn from an ear vein for sugar determinations, which were made in duplicate by the Folin-Svedberg[9] method. The sugar level was determined before etherization and every 15 minutes thereafter, during the administration of the ether, for one hour. A final analysis was made one hour after cessation of the anesthesia. The ether was administered carefully in order to avoid struggle and to minimize emotional reactions. As soon as relaxation was obtained a tube was inserted between the vocal cords into the trachea, and artificial respiration was started in order to avoid asphyxia. The depth of the anesthesia was regulated so that the corneal reflex was just present. Experiments were performed on normal cats, on cats with the left adrenal denervated, the right removed and the hepatic nerves cut,[5] and on cats completely sympathectomized.[10] The completeness of the sympathectomy was assured by noting absence of erection of the hairs on exposure to cold or during emotional excitement. Careful necropsy verified these findings.

The actual figures for a single experiment only are given in the chart, but each result has been fully confirmed. The well-known hyperglycemia of etherization is shown in Fig. 1A. If the adrenals

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[1] Reynoso, A., *Compt. rend. Soc. Biol.*, 1853, **5**, 116.

[2] Seelig, A., *Arch. f. exp. Path. u. Pharmakol.*, 1905, **52**, 481.

[3] King, J. H., Chaffee, B. S., Anderson, D. B., and Redelings, L. H., *Johns Hopkins Hosp. Bul.*, 1911, **22**, 388.

[4] Keeton, R. W., and Ross, E. L., *Am. J. Physiol.*, 1919, **48**, 146.

[5] Bulatao, E., and Cannon, W. B., *Am. J. Physiol.*, 1925, **72**, 295.

[6] Britton, S. W., *Am. J. Physiol.*, 1928, **86**, 340.

[7] Reid, C., *J. Physiol.*, 1932, **75**, Proc., 40.

[8] Cannon, W. B., and Bacq, Z. M., *Am. J. Physiol.*, 1931, **96**, 392.

[9] Folin, O., and Svedberg, A., *J. Biol. Chem.*, 1930, **88**, 85.

[10] Cannon, W. B., Newton, H. F., Bright, E. M., Menkin, V., and Moore, R. M., *Am. J. Physiol.*, 1929, **89**, 84.

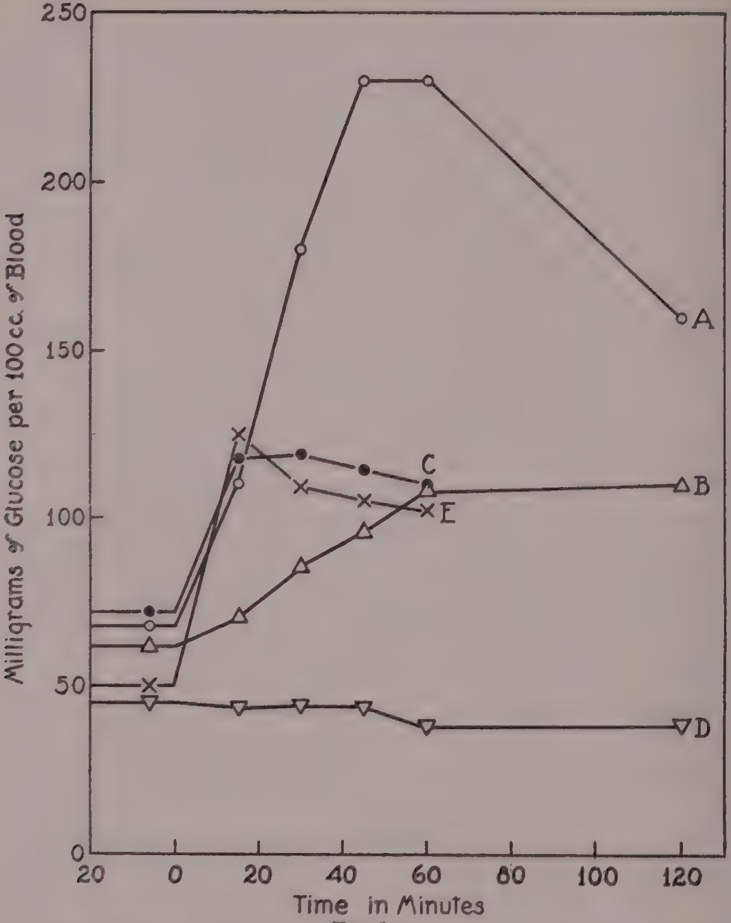

FIG. 1.

Adult cats fasted 24 hours previously. Ether administered from 0 to 60 minutes. A. Normal. B. Right adrenal removed, left adrenal denervated and liver nerves cut 10 days previously. C. Sympathectomy completed 3 weeks previously. D. Same preparation as C, but with adrenals removed aseptically 24 hours previously. E. Sympathectomy completed 2 weeks previously; adrenals removed 7 hours before etherization.

are inactivated and the liver nerves cut, this rise in blood sugar is considerably reduced (Fig. 1B). Sympathectomy does not abolish this increase in the glycemic level, as can be seen from Fig. 1C.

It is known that asphyxia can stimulate medulli-adrenal secretion directly, even if all connections of the adrenals to the central nervous system have been severed.[11] It seemed possible that the increase in blood sugar observed in the completely sympathectomized animal was due to the direct stimulating action of ether upon the adrenals. Both adrenals were therefore removed aseptically in the completely sympathectomized animal shown in Fig. 1C. Twenty hours later, ether again was administered for one hour and no rise in blood sugar was obtained (Fig. 1D). The low fasting blood sugar in this cat made us suspect that the liver might have been deficient in glycogen, a condition which has been reported after bilateral adrenalectomy.[12] Ether was therefore administered to a completely sympathectomized cat seven hours after bilateral adrenalectomy. An increase in blood sugar (Fig. 1E) was readily demonstrable.

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[11] Zwemer, R. L., and Newton, H. F., *Am. J. Physiol.*, 1928, **85**, 507.

The mechanism of the rise of blood sugar which takes place on etherization after ruling out the sympathico-adrenal factor is unexplained.

Summary. The elevation of the blood-sugar level in cats on etherization (Fig. 1A) is considerably reduced by inactivating the adrenals and cutting the liver nerves (Fig. 1B).

The residual increase persists in the completely sympathectomized animal (Fig. 1C), and therefore does not result from the production of sympathin.

The persistent hyperglycemia does not depend upon direct stimulation of adrenal secretion by ether since it is still present when both adrenals have been removed in the completely sympathectomized cat (Fig. 1E).

7096 C

Toxic Effect of Sodium Monoiodoacetate on Trypanosomes.

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From the Research Laboratories, Burroughs Wellcome and Co., Tuckahoe, N. Y.

It has been demonstrated by several investigators[1-7] that mono-iodoacetic acid will react with sulfhydryl groups, presumably according to the following equation—

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[12] Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, **100**, 693.

[1] Bersin, T., *Biochem. Z.*, 1932, **248**, 3.

[2] Dickens, F., *Nature*, 1933, **131**, 130.

[3] Quastel, J. H., *Nature*, 1933, **131**, 206.

[4] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 1932, **26**, 216.

[5] Waldschmidt-Leitz, E., Wieland, L. u. Purr, A., *Z. physiol. Chem.*, 1933, **215**, 64.

[6] Rapkine, L., *Compt. Rend. Soc. Biol.*, 1933, **112**, 790.

[7] Schroeder, E. F., Woodward, G. E., and Platt, M. E., *J. Biol. Chem.*, 1933, **101**, 133.

$$RSH + CH_2ICOOH \longrightarrow R\text{-}S\text{-}CH_2COOH + HI$$

That this reaction may have important biological applications is evident from the demonstration that the poisonous effect of iodoacetic acid on the action of the enzyme glyoxalase has been shown[2] to be due to the removal of the co-ferment, reduced glutathione.[8]

It is also known that some organic arsenicals react with thiol compounds forming condensation products,[9] and according to the theory of Voegtlin arsenicals exert their toxic effect upon trypanosomes by combining with sulfhydryl groups present in and, in his opinion, essential for the oxygen consumption of these organisms. If the above considerations are correct, it would seem to follow, necessarily, that iodoacetic acid must be toxic to trypanosomes.

In vitro experiments, carried out to test the above conclusion, showed that sodium iodoacetate added to *Trypanosoma equiperdum,* suspended in the citrated blood of a rat, is in fact very toxic. A 0.001 M solution killed all the organisms as a rule in 2 or 3 minutes. This time naturally varies with the number of trypanosomes in the emulsion and with their resistance. In view of this finding it seemed desirable to test the action of iodoacetate on trypanosomes *in vivo.*

Preliminary experiments with 14 rats showed that the maximum tolerated dose of sodium iodoacetate, injected intravenously into a normal 240 gm. rat, is 0.2 cc. of a 0.25 M solution. 0.3 cc. of this solution kills about 50% of the rats of this weight. These figures are in good agreement with the findings of Hall and Field[10] on the toxicity of intraperitoneally injected iodoacetate. Subcutaneous and intramuscular injections have also been tried. The toxicity was practically independent of the mode of injection. If rats, which have been infected with *Tr. equiperdum* and which contain in their blood stream at the time of treatment one trypanosome per 10-15 red blood cells, are injected with doses of 0.2 cc. of a 0.25 M solution, their blood becomes free of trypanosomes, usually in 2-3 hours. Some of the infected rats die from this dose, as a rule, some time after the trypanosomes have disappeared from the blood stream. Thus, it seems that the infected animals are somewhat more sensitive to iodoacetate than normal rats. The blood remains free of trypanosomes for a period varying from 2 to 10 days. Trypanosomes then reappear. Another dose of iodoacetate may be given and it again clears the blood stream for about the same length of time. This treatment has to date been repeated 7 times and the animals are apparently in good health with the exception of some inflammation and soreness at the place of injection (the tail). These results show that iodoacetate is also very toxic to trypanosomes *in vivo*. Furthermore, they indicate that compounds like iodoacetic acid offer some possibility of development to practical chemotherapeutic agents.

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[8] Lohmann, K., *Biochem. Z.*, 1932, **254**, 332.

[9] Voegtlin, C., Dyer, H., and Leonard, C. S., *Public Health Repts.*, 1923, **38**, 1882.

[10] Hall, V. E., and Field, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 360.

In regard to the mechanism of iodoacetate action, the results just quoted would appear to justify the prediction reached in the introduction. There are, however, differences in the action of iodoacetate and of arsenicals. Thus, the toxic action on trypanosomes *in vitro* could not be prevented or overcome by the addition of sulfhydryl in the form of cystein or thioglycollate solutions. Concentrations up to 0.02 M (a 20 fold excess) were used. Arsenicals are detoxified in analogous experiments. Similarly, the toxic effect on rats or on trypanosome infections in rats could not be prevented or overcome by the intravenous injections of these thiol compounds. This is again in contrast to the results with arsenic.[9, 11] If the conditions are comparable, our results are also somewhat in contrast to the results of Quastel and Wheatley,[4] who found that the inhibiting action of iodoacetate on glucose oxidation by brain tissue could be much decreased by the addition of cystein, glutathione or sodium thiosulphate.

Another point of difference between arsenicals and iodoacetate is the behavior of an arsenic fast strain of trypanosomes toward the 2 compounds. The concentration of neoarsphenamine which kills the resistant strain used in a certain time is about 16 times that of the concentration which kills the normal strain in the same time. Iodoacetate is, however, just as toxic to a resistant as to a normal strain. If both reagents exert their toxic effects on rat and on protozoon solely by combining with thiol groups, the results indicate that the reactions are different in some respects.

Schroeder, Woodward and Platt[7] have stated that the action of iodoacetate on yeast is not due solely to combination with thiol groups. There is also some evidence that this is true for muscle. Thus, Lohmann[12] has shown that its efficiency in inhibiting lactic acid production from glycogen is far greater than its efficiency in inhibiting glyoxalase action. He has also shown that the former

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[11] Reiner, L., and Leonard, C. S., *Arch. Int. Pharmacodynamie et Therap.*, 1932, **43**, 49.

[12] Lohmann, K., *Biochem. Z.*, 1933, **262**, 152.

effect remains after a muscle extract has been dialyzed free of glutathione and cannot be removed by the re-addition of glutathione. Maschmann and Helmert[13] have found that the enzymes, kathepsin and papain, are both inhibited by iodoacetate and cannot be reactivated by thiol compounds. Bersin and Logemann[14] have obtained the same results with papain. Meyerhof and Kiessling[15] have presented evidence that one effect of iodoacetate on muscle is to prevent the reaction of pyruvic acid and glycerine phosphate. They also add further evidence to the finding that the effect of sodium fluoride on muscle is to prevent the formation of pyruvic acid from glyceric acid monophosphoric ester. It seemed of interest, therefore, to test the toxicity of sodium fluoride to trypanosomes *in vitro*. It was found to be more than 100 times less toxic than iodoacetate.

As a general conclusion from these experiments, it seems that if the iodoacetate reacts with thiol groups in trypanosomes either the result of this is different from the supposed action of arsenic with these groups, or the iodoacetate, in addition to its reaction with thiol groups, acts by another mechanism.

Summary. 1. Sodium monoiodoacetate is highly toxic to *Trypanosoma equiperdum*. 2. The blood stream of rats infected with trypanosomes can be freed from these organisms by the intravenous injection of tolerated doses of sodium iodoacetate. The blood remains free for a period varying from 2 to 10 days after which trypanosomes reappear. The treatment may be repeated. 3. The toxic action of iodoacetate upon either rats or trypanosomes could not be counteracted by a 20-fold excess of thiol compounds. 4. Arsenic resistant strains of trypanosomes are not resistant to iodoacetate. 5. The mechanism of the action of iodoacetate is discussed.

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[13] Maschmann, E., u. Helmert, E., *Z. physiol. Chem.*, 1933, **220**, 199.

[14] Bersin, T., u. Logemann, W., *Z. physiol. Chem.*, 1933, **220**, 209.

[15] Meyerhof, O., u. Kiessling, W., *Biochem. Z.*, 1933, **264**, 40.

7097 P

Pathological Changes in the Dog Kidney Resembling Normal Histological Structure in the Aglomerular Fish Kidney, Opsanus Tau.*

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From the Laboratory of Pharmacology, the University of North Carolina.

During the past 10 years investigations[1, 2, 3] have been in progress in this laboratory which have been concerned with the acute changes of a degenerative character which may be induced in the dog kidney by the use of suitable subcutaneous injections of uranium nitrate, and with the histological changes arising as processes of repair in the kidneys of animals which survive such injections that result in the development of a pathological state which may be designated a chronic nephritis.

This preliminary report is based on the study of the kidneys of 6 dogs with a chronic nephritis developing from the use of uranium, the duration of which has varied from 4 years to 6 years and 8 months. In addition to the usual routine functional studies which have been made of the blood and urine of these animals, histological studies have been undertaken of biopsy material removed from the kidneys before the commencement of the uranium intoxications, during the acute degenerative phase of the renal injury and at intervals of 3 months during the periods of repair which have resulted in the establishment of a chronic nephritis.

The biopsy material obtained for study has been in the form of wedge shaped pieces of tissue taken from both poles and the outer convex border of the kidneys. Such material has represented a cortical surface 1-1.5 cm. in width with a depth extending into the substance of the kidney of 0.5-1.5 cm. The exposed renal surfaces caused by the removal of such material were closed with cat gut chromicised sutures. The tissue was fixed in corrosive-acetic, cut in serial sections and stained with haematoxylin and eosin.

The primary injury to the kidney from uranium, as has been emphasized by Suzuki,[4] is to the convoluted tubule epithelium. The processes of degeneration and repair to this tissue have been de-

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* This investigation was made possible by the Edward N. Gibbs Prize Fund of the New York Academy of Medicine.

[1] MacNider, W. deB., *J. Exp. Med.*, 1929, **49**, 384.

[2] MacNider, W. deB., *J. Exp. Med.*, 1929, **49**, 411.

[3] MacNider, W. deB., *Science*, 1931, **73**, 103.

[4] Suzuki, L., *Morphologie der Nierensekretion*, Jena, 1912.

scribed.[1, 2] The glomerular injury which is delayed in its development, results during a period of years in inducing either a partial or complete structural obliteration of these structures so that areas exist in the kidneys of such animals, as shown in serial sections, in which the glomeruli are either destroyed by a process of connective tissue formation with a secondary hyalinization or they exist as relatively avascular units in which one or more capillary loops remain open for blood distribution.

In certain areas the largely hyalinized glomeruli give the appearance of having become canalized for the passage of blood. In such spaces blood cells with normal staining reactions can be demonstrated. In such zones of glomerular degeneration and obliteration, the epithelium in the convoluted tubule areas, though changed morphologically, has shown an unusual degree of preservation. Such tubules have become separated by an increase in intertubular connective tissue in which is found a degree of vascularity which can not be explained as arising from an inflow of blood through those glomeruli which may yet show some degree of patency. Furthermore, the study of serial sections made from biopsy material from the kidneys of these animals during the fourth, fifth, and sixth years of the existence of a chronic nephritis has shown areas in which the glomeruli have undergone complete obliteration, and yet in such zones a modified type of convoluted tubule epithelium has been preserved. Such areas resemble the normal structure first described by Marshall[5] for the aglomerular fish, *Opsanus tau.*

The source of the intertubular blood supply in such aglomerular areas and in zones, the seat of advanced glomerular degeneration is now the object of investigation in which the whole kidney is studied following appropriate methods of injection. In serial sections now available, vessels may be traced growing into the renal cortex from the medulla, which apparently arise from those vessels which take their origin from the arcuate arteries and passing down into the medulla furnish this tissue with a portion of its blood supply.[6] On the cortical side of such areas there has occurred an ingrowth of vessels through adhesions formed between the surface of the cortex and surrounding tissue, the formation of which resulted from the stripping of the kidney from its bed of perirenal fat and the operative injury necessitated by the removal of biopsy material.

These observations suggest 2 possibilities: (1) that an organ, the seat of processes of degeneration followed by repair, may as a result of the latter process revert back to a type of structure normal for a remote ancestral form; (2) the possibility of inducing an ingrowth of vessels into the kidney through the cortex in an attempt to maintain function in a relatively aglomerular structure.

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[5] Marshall, E. K., Jr., *Bull. Johns Hopkins Hosp.*, 1929, **45**, 95.

[6] MacNider, W. deB., *J. Med. Research*, 1911, **19**, 425.

7098 P

Further Studies on the Cultivation of Mycobacterium Leprae.

EARL B. MC KINLEY AND ELIZABETH VERDER.

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, George Washington University.

We[1] have previously reported the cultivation of *Mycobacterium leprae* in minced chick embryo suspended in Tyrode's solution direct from leprous nodules containing acid-fast organisms. Evidence of growth and multiplication was obtained in as few as 5 days although subsequent experience has shown that better growth occurs in from 10 days to 2 weeks. The leprosy nodules were digested with 3% sodium hydroxide to free the organisms from the tissue and to destroy contaminants and, following neutralization, the tissue medium was inoculated. Human embryonic tissue (spleen, liver, lung) has also been employed and more experience with this tissue leads us to believe that it is a better medium than chick embryo, contrary to our previous report, although it is, of course, more difficult to obtain.

Leprosy nodules which have been treated as described above, the acid-fast organisms from which have been seeded in minced embryonic tissue suspended in Tyrode's solution, have been found to contain acid-fast organisms which are unquestionably alive. After carrying such cultures in the tissue medium through several generations we wished to test the viability of these acid-fast organisms by placing them on a solid medium, such as the hormone glycerol agar, under a gaseous tension of CO_2 and oxygen. Such cultures were prepared and were incubated under these conditions for 3 months. In one series of 10 cultures of this type we have found 6 to be positive as judged by definite micro-colonies of acid-fast organisms (quite similar to those which have been described for *B. tuberculosis* obtained from the blood stream) present in smears from the cultures when such smears are taken from suspicious areas which can be identified grossly. These micro-colonies are somewhat difficult to demonstrate and require a perseverance not usually required in the ordinary casual examination of a bacteriological smear, but their identification is definite and, we believe, represents evidence that the acid-fast organisms in the tissue culture are viable after several generations in such mediums. The demonstration of these micro-colonies may prove to be significant in offering an explanation of the frequent failure to demonstrate acid-fast organisms in the very early lesions of leprosy.

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[1] McKinley, E. B., and Verder, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 659.

Further study of the chick embryo tissue method of cultivation of the acid-fast organisms from leprosy tissue indicates that multiplication does not continue to improve with each subculture. The medium, as has been reported, tends to become quite alkaline and, while the organisms appear to grow better in the beginning they later slow up and this may be due to the reaction of the medium. The same is apparently true with *B. tuberculosis* bovine, somewhat less so with human *B. tuberculosis*, though avian *B. tuberculosis* continues to grow luxuriantly.

The method of growing various strains of acid-fast organisms in minced embryonic tissue suspended in Tyrode's solution (or in ordinary broth which has given good results in a limited number of trials) deserves further study. It is possible that such cultures might be decidedly improved if they were placed in a gaseous tension as described by one of us with Soule.[2, 3, 4] We feel that this method may eventually be employed under such favorable conditions as to give most promising results with the group of acid-fast organisms.

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[2] McKinley, E. B., and Soule, M. H., *J. Am. Med. Assn.*, 1932, **98**, 361.

[3] Soule, M. H., and McKinley, E. B., *Am. J. Trop. Med.*, 1932, **12**, 1.

[4] Soule, M. H., and McKinley, E. B., *Am. J. Trop. Med.*, 1932, **12**, 441.

7099 P

Experimental Observations on the St. Louis Epidemic of Encephalitis.

EARL B. MC KINLEY AND ELIZABETH VERDER.

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, George Washington University.

Through the kindness of Dr. E. V. Cowdry and Dr. Ralph S. Muckenfuss of the School of Medicine, Washington University, we have been permitted to study brain tissue from 2 fatal cases of the recent epidemic of encephalitis in St. Louis. When received by us, these 2 brains were immediately cultured anaerobically and aerobically in several different mediums to determine the presence or absence of visible microorganisms, such as the streptococcus. All cultures *were entirely negative.* Emulsions prepared with these 2 brains were then injected intracerebrally into a large number of different animals, including Rhesus and Cebus monkeys, rabbits, guinea pigs, kangaroo rats, white rats and mice, canaries, pigeons, ferrets, dogs, puppies, cats and kittens. None of these animals or fowls inoculated has developed any symptoms definitely characteristic of encephalitis after 47 days. On the twenty-fifth day following inoculation one of the kittens died with what appeared to be cerebral involvement, but upon autopsy, and after examination of sections of the brain, no *evidence of encephalitis was demonstrable.* On the twenty-seventh day one of the kangaroo rats died, but this brain was also negative. Later a pigeon died, which was negative for any suspicious lesions and on the thirty-eighth day a Cebus monkey died, the brain of which at first looked suspicious, but later it was decided that we had little, if any, evidence of encephalitis. Cultures of this brain, however, have shown a luxuriantly growing staphylococcus to be present in the tissue, though direct smears from the surface of the brain and pipettings of the brain substance are negative for this organism. This organism is regarded only as a secondary invader as we[1, 2] have emphasized in previous work in regard to green streptococci when these organisms occur in the brains of such animals. Even with the presence of this organism in the central nervous system at death the absence of definite pathology of an encephalitis is noteworthy and confirms the interpretation placed upon its presence as a secondary invader.

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[1] McKinley, Earl B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 436.

[2] McKinley, Earl B., and Douglass, M., *J. Infect. Dis.*, 1930, **47**, 511.

That the above explanation is the true one is emphasized further by the fact that cultures from the brains of other animals dying during these experiments have been entirely negative. Furthermore, the other monkeys have had no symptoms referable to a central nervous system involvement and one Rhesus monkey, which was sacrificed 18 days following inoculation, gave negative cultures from both brain and heart blood.

The 2 brains received from St. Louis were preserved in glycerol. This raises the question of the bactericidal effect of this method of preservation. It is beginning to appear that we must modify our former concepts regarding this matter. The real benefit to be derived from storing virus-infected tissues in glycerol is to retard autolysis of tissue cells, the end products of which appear to be detrimental to filterable viruses. That glycerol may destroy, or at least inhibit, certain gross bacterial contamination in tissues has long been recognized, but its killing effect has been much over emphasized. We have on several occasions isolated green streptococci or staphylococci from virus-infected brains of experimental animals following storage in glycerol and one authority has informed us that he has preserved the viability of green streptococci for as long as 4 years in glycerol. Francis[3] has stated that he has maintained viability and virulence of *B. tularense* in 100% glycerol for over 6 years and *B. pestis* for over 7 years, when the glycerinated tissues are kept in the cold. Glycerol is a more effective bactericide, of course, if allowed to act at room or higher temperature. It would appear then that cultural data based upon tissues stored for relatively short periods in glycerol or under conditions of low temperature for very long periods are not invalidated because of the erroneous concept of the efficiency of glycerol as a bactericide.

While our study of these 2 cases has not led to the establishing of any causative agent of the St. Louis epidemic of encephalitis in laboratory animals we feel that new and convincing evidence has been found confirming our earlier work that no known bacterial incitant is involved in these cases. The St. Louis epidemic is somewhat different from previous epidemics reported in this country and Europe, though similar to those described in Japan and in Australia. We are not certain whether these epidemics are all produced by the same agent (such as a filterable virus) or not. A study of the epidemiology and character of these various epidemics brings to mind again the possibility of their relation to epidemic influenza of probable filterable virus origin which, in some epidemics, may be of gastrointestinal nature, in others upper respiratory and possibly in others one, or both, of these clinical pictures with the predominating effects soon after onset manifest in the central nervous system, the pathology being that of an encephalitis. The clinical history of this disease, the lack of discovery of the true etiological agent over these many years, permits at least the raising of the question again as to its possible specific relation to epidemic influenza, most probably caused by an ultramicroscopic virus.

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[3] Francis, Edward, personal communication, 1933.

7100

Assay with the Guinea Pig of the Lactogenic Hypophyseal Hormone.*

W. R. LYONS AND H. R. CATCHPOLE. (Introduced by Herbert M. Evans.)

From the Division of Anatomy, University of California.

Corner's[1] results on mammary stimulation in virgin ovariectomized rabbits by injections of hypophyseal extracts were confirmed by Nelson and Pfiffner[2] in guinea pigs. They obtained lactation in 3 ovariectomized, virgin guinea pigs following injections of hypophyseal extracts alone.

In our own investigations of the lactogenic hypophyseal hormone,† rats, rabbits, guinea pigs, cows, dogs, a monkey and an opossum have been used. Lactation was readily induced in all forms except the rat, which was poorly responsive. We have also used the squab test‡ of Riddle *et al*[3] for lactogenic hormone on some hun-

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* Aided by a grant from the Rockefeller Foundation. This fund has been generously augmented by the Board of Research and by the College of Agriculture of this University.

[1] Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.

[2] Nelson, W. O., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **25**, 1.

† Lactogenic hormone has been prepared by making acid-acetone extracts (approx. 7.0 cc. conc. HCl were added to 1 liter of 66% acetone) of acetone-dried anterior pituitary powders from cattle, pigs and lambs. The active principle is precipitated out of the cleared extract at a concentration of acetone between 83-90% at a pH of about 2.0. This white precipitate represents a crude lactogenic fraction and is free of gonadotropic hormone, but may sometimes be slightly contaminated with growth hormone.

By isoelectric precipitation, powders readily soluble in water-clear salt solutions at pH 6.0 and 7.0, but insoluble at 6.4 have been obtained, which were active for dred birds. While satisfied with it, more particularly because the test animals may be used for simultaneous assay of other anterior pituitary hormones, we have nevertheless been interested in using the guinea pig as a favorable mammalian form in which to study mammary response. We have observed lactation in more than 100 virgin guinea pigs (600-700 gm.) ovariectomized in oestrus and injected immediately thereafter with lactogenic hormone. Such animals, contrary to Riddle's[3] opinion, are very reactive without previous injections of ovarian hormones (the influence of preceding ovarian cycles probably suffices to "prepare" the gland). Virgins in oestrus are chosen because at this time of the cycle a few drops of clear, watery fluid containing colostrum-corpuscles may be expressed from the gland, indicating that the gland is at the height of its cyclical growth.[4] To date we have never observed milk secretion in normal or ovariectomized virgin guinea pigs uninjected with the lactogenic hormone. As early as 8 hours following the injection of the lactogenic principle, the serous secretion may be replaced by a turbid one; and it has been possible following potent hormonal injections to express milk in streams within 24 hours.

It is preferable to remove the ovaries of the test animal, especially if there is a suspicion that the extracts being tested contain the gonadotropic factor. It is very difficult even at this time to ascertain which of the mammary reactions in non-castrates reported in the earlier literature were due to injected lactogenic hormone and which to the test animal's own hypophyseal lactogenic hormone released because of induced ovarian conditions. That the gonad-stimulating hormone of the anterior lobe is not in itself responsible for direct mammary stimulation has been definitely shown.[3, 5, 6]

It is possible in the guinea pig to grade the mammary response by judging the spreads as Gardner and Turner[5] do with the rabbit, but this has little advantage over the expression of milk by manipulation.

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lactogenic hormone when tested on squabs and guinea pigs in doses of less than 1 mg. and which were negative for the growth and gonadotropic hormones.

[3] Riddle, O., Bates, R. W., and Dykeshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

‡ In this test we believe that one need not depend upon a weight increase in the crop-gland over and above the control, to determine a positive reaction, since beginning growth changes may be seen in crops that weigh less than the average normal, as early as 48 hours after the injection of potent hormone. Stained spreads may be made of these crops just as is done with mammary gland, although a qualitative reaction may be recognized by merely holding them to the light.

[4] Loeb, L., and Hesselberg, C., *J. Exp. Med.*, 1917, **25**, 285.

[5] Gardner, W. W., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1933, 196.

[6] Catchpole, H. R., and Lyons, W. R., *Anat. Rec.*, 1933, **55**, 49.

Both are qualitative tests, but the minimal effective dose may be arrived at quite as easily by qualitative methods as quantitative. With further purification of the lactogenic hormone it may be possible to use guinea pigs repeatedly for tests, as is done in assays of oestrin. We have on several occasions induced a second lactation 2 weeks after an earlier positive reaction had subsided in ovariectomized virgins. On the other hand, with crude lactogenic extracts of beef hypophysis we have observed anaphylactic death following the second injection.

7101

Induction of Lactation in Heifers with the Hypophyseal Lactogenic Hormone.*

H. R. CATCHPOLE, W. R. LYONS AND W. M. REGAN.
(Introduced by Herbert M. Evans.)

From the Divisions of Animal Husbandry at Davis and Anatomy at Berkeley, University of California.

We present here results of preliminary experiments on the possibility of producing lactation in the virgin heifer by the injection of the hypophyseal lactogenic hormone. A 1% solution of crude lactogenic hormone† prepared from bovine anterior lobes was used. We employed 2 Holstein heifers 16 months old and 2 Ayrshire heifers 12 months old.

Holstein Experiments. The udders of these animals H-1 and H-2 were manipulated twice daily for 1 week. At the end of this time H-1 showed a very slight serous secretion. 10.0 cc. of the hormone were then injected subcutaneously daily for 11 days into this animal. The secretion became milky on the fourth day of injection and increased rapidly in amount up to 500 cc. daily by the eleventh day when milking was stopped and the heifer allowed to go dry. Through the courtesy of Dr. G. A. Richardson of the Division of Dairy Industry, University of California at Davis, samples of the tenth and eleventh days secretions were analyzed. The fat content, pH, and co-

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* Aided by a grant from the Rockefeller Foundation. This fund has been generously augmented by the Board of Research and by the College of Agriculture of this University.

† This preparation was free of gonad-stimulating hormone (immature rat test) but gave rise to reducing substances in the urine of injected dogs. For method of preparation see accompanying note by Lyons and Catchpole.

agulation times of the milks were within the usual limits of variation. Without knowledge of the source, the flavor was criticized as "not definitely pleasing". The most prominent taste was salty, due to low lactose and high chloride content. The consistency of the milk was somewhat thick, probably due to the high protein content. Peroxidase was present. It was agreed that in the course of 10 days the hormone had induced a secretion at least sensibly approaching normal constitution, and we would expect the discrepancies to vanish should the milk secreting mechanism get fully under way with continued treatment.

The control animal H-2 was manipulated daily for 22 days. A few drops of a salty, serous fluid could be expressed on the 15th day and after. Beginning on the twenty-second day, a total of 100 cc. hormone was injected over a period of 13 days. By the tenth day the secretion was milky in appearance. On each of the fourteenth and fifteenth days about 250 cc. of milk were obtained. Milking was continued until the eighteenth day, when the secretion had begun to fall off in amount, since hormone was no longer being given.

Ayrshire Experiments. The results obtained with these animals, A-1 and A-2 gave important leads for further experimentation. A-2 was used as a control animal for the first 21 days. Manipulation during this time gave nothing beyond a few drops of serous, salty fluid. A total of 115 cc. hormone was then given over a period of 14 days. The secretion became milky on the fifth day and increased in amount to a 300 cc. yield on the sixteenth day. By the nineteenth day with continued milking (injections having stopped on the fourteenth day) the yield fell off as in the case of H-2 above.

The heifer, H-1 showed an udder development rather less than any of the other experimental animals, and injection of a total of 150 cc. of hormone over 22 days provoked no response. Even the slight serous secretion was absent. This cow was in heat on the twentieth day of the experiment and on the twenty-third day a scanty serous secretion appeared. A further 100 cc. hormone injected over the next 12 days led to the production of 80 cc. milk by the fourteenth day. This fell off on ceasing the injections.

The results with the heifer A-2 may be viewed in the light of our finding in the rabbit that the mammary apparatus of the sexually immature animal is apparently insensitive to the lactogenic hormone.[1] Preparation of the mammary gland with an ovarian factor seems essential for stimulation by the lactogenic hormone. Whether in the heifer the degree of mammary sensitiveness to lactogenic hormone action is normally determined by the number of cycles that the animal has passed through will be determined by further experiments with heifers at these borderline stages.

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[1] Catchpole, H. R., and Lyons, W. R., *Anat. Rec.*, 1933, **55**, 49.

A comparison of our data with those of E. I. Evans[2] makes it appear that our heifers were just at, or just over the borderline of mammary reactivity. This author does not, however, state the ages of the virgin heifers that were used in his experiment.

7102

Experiments with Hypophyseal Lactogenic Hormone on Normal Ovariectomized and Hypophysectomized Dogs.

W. R. LYONS, I. L. CHAIKOFF AND F. L. REICHERT.

From the Divisions of Anatomy and Physiology, University of California Medical School, Berkeley, and the Halstead Laboratory of Experimental Surgery, Stanford University Medical School.

The present report deals with the effects of the lactogenic hormone in normal dogs and in dogs following the removal of ovaries and hypophysis. The hormone used in this investigation was obtained in powder form from an acid-acetone extract of the anterior pituitary.

One mg. of the dry powder was equivalent to 40 mg. of fresh anterior lobe. Gonad-stimulating hormone could not be demonstrated in 100-mg. doses of such powders tested on immature pigeons and rats. No attempt was made in these experiments to free these powders of some slight contamination with growth hormone, although potent isoelectric precipitations at pH 6.4 have yielded growth-free lactogenic hormone. Full details of the method of preparation are submitted separately.

The results are shown in tabular form. It was found that 2 subcutaneous injections representing a total of 20 mg. of crude lactogenic hormone sufficed to cause the secretion of milk in normal parous and non-parous mature bitches.

Lactation was obtained in 3 bitches ovariectomized one day previous to administration of the hormone.

The hormone induced lactation in a single bitch hypophysectomized one week prior to treatment. The mammary glands of this animal were well developed and did not yet show regressive changes

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[2] Evans, E. I., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 1372.

TABLE I.
Reaction of Dogs to Injection of the Lactogenic Hormone.

Designation of dog	History	Mammaries	Injections	Mg. Powder Injected	Mg. Powder Effective	Result
1	Littered 10 mo. previously	Resting No milk	6	60	60	Lactation
2	Weaned litter 1 wk. previously	Regressing No milk	10	200	20	Returned to full lactation
4	Non-parous. Post-oestrum	Resting No milk	7	140	40	Copious lactation
7	Weaned litter 1 mo. previously	Resting No milk	7	140	80	Lactation
12	Mature bitch Dioestrum	Resting No milk	6	120	40	Copious Lactation
6	Mature bitch ovariectomized 1 day previously (Dioestrum)	Resting No milk	8	160	120	Lactation
5	Parous. Post-oestrum, ovariectomized 1 day previously	Well-developed. No milk	3	60	40	Copious lactation
1	Parous. Dioestrum. Ovariectomized 1 day previously	Resting No milk	8	160	120	Lactation
13	Parous, Hypophysectomized 1 week previously	Well-developed. No milk	6	120	40	Copious Lactation
14	Senile. Hypophysectomized 1 month previously	Atrophic. No milk	10	200	—	No effect on mammaries
15	Immature. Hypophysectomized 2 weeks previously	Infantile No milk	11	310	—	No effect on mammaries

as the result of hypophysectomy. Two other hypophysectomized dogs failed to respond, although several times the amount of the hormone found necessary to induce lactation in normal dogs was injected in these animals. The failure, however, to obtain lactation with the hormone in these 2 dogs may probably be ascribed to lack of mammary development, inasmuch as one of these animals was sexually immature with infantile mammae, while the other was a senile bitch with atrophied mammae and ovaries.

All dogs in which lactation was induced continued secreting milk for about 2 weeks after the last injection. Even a non-parous bitch was stripped of approximately 100 cc. of milk on 3 consecutive days. A mother that had been removed from her litter and had been dry for a week was brought back to full lactation to continue suckling.

The response of completely depancreatized dogs to the lactation hormone has been reported elsewhere.[1] It is interesting to note in this connection that no secretion of milk was observed in 5 depancreatized dogs following injections of the hormone, whereas in a single dog lactation was obtained.

7103 C

Availability of the Rabbit for Assay of the Hypophyseal Lactogenic Hormone.*

W. R. LYONS AND H. R. CATCHPOLE. (*Introduced by Herbert M. Evans.*)

From the Department of Anatomy, University of California.

Two years ago, while investigating various preparations of the anterior pituitary for their lactogenic effect we employed castrated virgin rabbits, as had Corner[1] before us. While satisfied with the results in the rabbit, we prefer at present to use the virgin guinea pig ovariectomized in oestrus and injected immediately thereafter (Nelson and Pfiffner[2]) or the 4 to 5 weeks old squab (Riddle[3]) as more convenient forms. It would seem, however, that the same degree of satisfaction with the castrate virgin rabbit has not been experienced by some other investigators. This report restates our position on the action of the lactogenic hormone in rabbits, and submits further information concerning our experiences with this animal.

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[1] Chaikoff, I. L., and Lyons, W. R., *Am. J. Physiol.*, in press.

* Aided by a grant from the Rockefeller Foundation. This fund has been generously augmented by the Board of Research and by the College of Agriculture of this University.

[1] Corner, G. W., *Am. J. Phys.*, 1930, **95**, 43.

[2] Nelson, W. O., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 1.

[3] Riddle, O., Bates, R. W., and Dykeshorn, S. W., *Am. J. Phys.*, 1933, **105**, 191.

We have reported[4] that a mature mammary gland in a virgin rabbit normally matured, then castrated, will respond by growth and lactation to the lactogenic hormone, but will merely atrophy following administration of potent growth hormone, or of potent gonadotropic hormone from pregnant mares' serum, or pregnant women's urine. That no continuing ovarian factor plays a rôle in the resultant growth of the ducts and alveoli of such positive reactions seems plausible since we always remove the ovaries just before beginning injections. Should ovarian secretions remain in the body after castration, their influence on the mammary apparatus could only be a synergistic one with the lactogenic hormone, since without administration of the latter the castrate rabbit's mammary glands gradually atrophy.

The rabbits used by us have been 5½ to 6 months old virgin New Zealand Whites and Reds weighing around 3 kg. If the nipples of these just mature females show any appreciable growth over and above the immature or male-sized nipples, then lactation may be expected following treatment with lactogenic hormone. Precaution must be exercised to ascertain that the animal is not already secreting milk. Stripping of the glands throughout their extent and observation of a control biopsy spread assures one on this point. We have often observed "spontaneous" lactation in virgin rabbits isolated from males and other females, but we have never found anything but atrophy of the mammary glands of untreated castrate rabbits not lactating at the time of operation. Spontaneous lactation has been observed in young virgin rabbits, the ovaries of which upon serial sectioning showed no evidence that a corpus luteum had ever been formed (although a considerable amount of interstitial tissue around atretic follicles was present), and such animals have been observed to lactate continuously for a month following removal of the ovaries, apparently in response to lactogenic hormone from the animals' own hypophyses.

For uniform results with the lactogenic hormone in rabbits one should use virgins, the ovaries of which show no evidence of corpus luteum formation. The ovaries of a just mature rabbit previously

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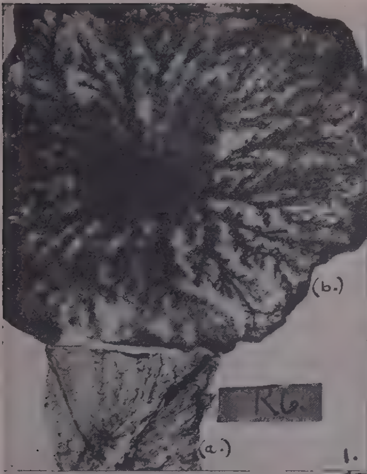
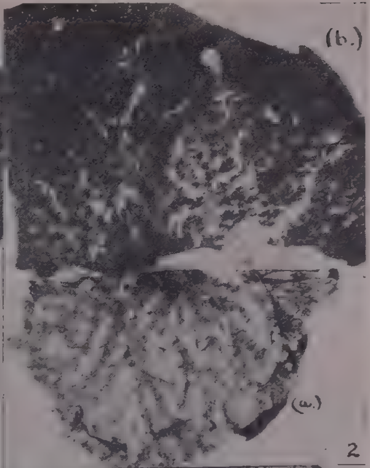
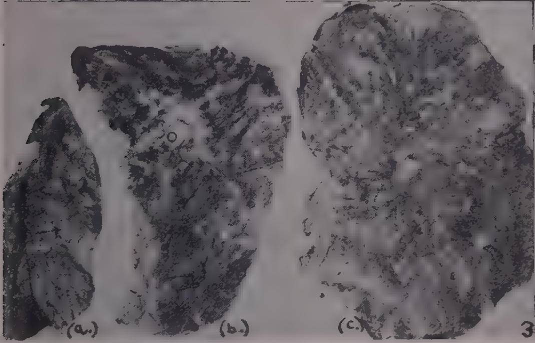
[4] Catchpole, H. R., and Lyons, W. R., *Anat. Rec.*, 1933, **55**, 49.







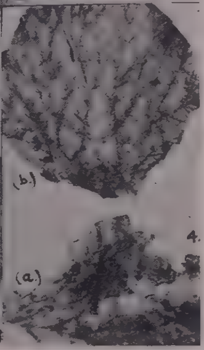


FIG. 1. Shows the control mammary spread (a) and the reaction (b) to the lactogenic hormone. This rabbit lactated after 7 daily injections (each 5.0 cc. of a 1% solution) of an acetone-ammonia preparation (sheep anterior lobes) free of growth but contaminated with the gonadotropic hormone.

FIG. 2. Shows the control spread (a) and the reaction (b) to two daily injections (each 5.0 cc. of a 1% solution) of a preparation similar to above.

FIG. 3. Shows the control spread (a) and the reaction (b) (lactating) after 5 daily injections (each 2.0 cc. of a 1% solution) and the reaction (c) to 5 more injections (each 5.0 cc. of a 1% solution) of an acid-acetone preparation (bovine anterior lobes) free of gonadotropic but slightly contaminated with growth hormone.

FIG. 4. Shows the control spread (a) and the negative reaction (b) to 12 daily injections (each 5.0 cc. of a 1% solution) of a preparation potent for growth but free of gonadotropic and lactogenic hormones.

isolated at weaning should show a crop of fairly large follicles but no corpora. Typical mammary spreads of such rabbits may be seen in the (a) parts of Figs. 1-4. Fig. 2a shows a mammary gland more developed than the others, but still not lactating. We feel that such glands (esp. 1, 3 and 4) are from rabbits just over the borderline of mammary (and sexual) maturity. Since in our hands such slightly developed glands respond to the lactogenic hormone alone by the growth shown in the (b) parts of Figs. 1-3, we should be inclined to stress a physiological rather than a morphological definition of mammary maturity by defining a mature mammary apparatus in the rabbit as one that is capable of responding to the lactogenic hormone by duct and alveolar growth, and by secretion of milk.

Such a statement is at variance with that made by Riddle *et al*,[3] who state that they have become "fully convinced that prolactin[†] has not in the least favored the growth and development of mammary tissue in the particular individuals with which we have worked," (guinea pigs and rabbits); and that of Gardner and Turner,[5] who state that "growth of the mammary gland in the rabbit has never been stimulated, at least to any marked degree, by injection of pituitary extracts." also that "Galactin[†] was ineffective in stimulating the growth of mammary glands of young, ovariectomized rabbits." When our photographs are compared with those of Gardner and Turner (loc. cit., Plate III, p. 26) it becomes apparent that we have produced growth as well as lactation in glands considered by them as non-reactive or infantile. With the establishment of a proper lactogenic hormone unit such differences in results will probably be cleared up.

We would suggest that if the rabbit is to be used as an assay animal rather than the squab or the castrated virgin guinea pig, it would consume less time and allow for less chance of error if mature, non-ovulated, non-lactating virgin rabbits castrated just prior to testing were used. It may be objected that ovariectomy need not be done, but a warning seems timely that solutions containing no lactogenic factor but rich in gonad-stimulating hormone may set up a condition in the ovary simulating that in pseudopregnancy, and lactation frequently follows this condition in the rabbit apparently through secretion of lactogenic hormone by the animal's own hypophysis. Consequently although Gardner and Turner[‡] use the pseudopregnant rabbit as a basis for their assay, we consider it difficult to differentiate between the effects caused by injected substances and those induced by the pseudopregnant state itself on the mammary glands of the rabbit.

[†] Names employed for the hypophyseal lactogenic hormone.

[5] Gardner, W. W., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 196, (1933).

[‡] These workers use 12-16 day pseudopregnant rabbits made pseudopregnant by injection of gonadotropic substances, or by sterile coitus. They treat with the lactogenic hormone solution for 7 days. According to their method a +++ or a ++++ but not a ++ reaction identifies the unit.

7104 P

Effect of Castration on Fasting Ketonuria in Rats Following Diacetic Acid Administration.*

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Deuel and Gulick[1] have shown that women are much more susceptible to fasting ketosis than are men. Although no sexual difference was observed by Butts and Deuel[2] in the level of the slight ketonuria which develops in rats during fasting, it was noted that the administration of diacetic acid by stomach tube to fasting rats in proportion to their surface areas was followed by a greater excretion of acetone bodies in the urine of the females than in that of the males. Similar results were obtained with guinea pigs.

In the present investigation a comparison has been made in the acetone body excretion in normal male and female rats with castrated ones of both sexes, some of which received theelin after the administration of diacetic acid. The experimental technic was similar to that employed in the earlier work.[2] Rats which were 4 to 5 months old were used. The number of rats used in each group is as follows: normal males, 24; castrate males, 12; castrate males with theelin, 9; normal females, 23; castrate females, 12; and castrate females with theelin, 9.

The acetone body excretion was uniformly about twice as high in the female as in the males. Castration in the male resulted in a small decrease in the level of ketonuria. However, the acetone body excretion in the oöphorectomized female rats was reduced to a value about one-fourth that of the normal females and about one-half that of normal males. This level showed no tendency to rise during the 5 days during which the experiment was carried on. The administration of 3 units of theelin per 140 gm. rat to castrate male and female rats daily for 3 days prior to and for the 5 days during which the diacetic acid was fed did not appreciably alter the ability of these animals to burn diacetic acid. This dose of theelin was sufficient to maintain the female animals in active oestrus during the entire experiment. In one series of tests, 10 times this amount of theelin was given also with negative results. It is concluded that the greater ketosis which obtains in females than in males is to be traced to some substance produced by the ovary other than theelin.

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* This work was aided by Grant 289 of the Committee on Scientific Research, American Medical Association.

[1] Deuel, H. J., Jr., and Gulick, M., *J. Biol. Chem.*, 1932, **96**, 25.

[2] Butts, J. S., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1933, **100** 45.

7105

Relation of Anterior Pituitary to Sexual Differences in Ketosis in the Rat.

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Burn and Ling[1] have reported that the administration of an alkaline extract of the anterior pituitary gland to female rats fed on a butter fat diet brought about a marked rise in the level of ketonuria. The experiments on males were uniformly negative. In order to determine whether the sexual differences in ketonuria reported in the previous paper[2] might be traced directly or indirectly to this ketogenetic hormone, further experiments under varying conditions seemed advisable.

The experimental procedure was similar to that used in the previous work.[2] The extracts of anterior pituitary were made from fresh glands of steers according to the procedure of Burn and Ling.[1] The administration of this extract to normal or castrated male or female rats which were receiving diacetic acid resulted in a marked rise in the level of acetone bodies in the urine over that of the controls. The response was as prompt and as great in male as in female rats and in the castrated as the normal animals. No glycosuria was observed. The following is the number of experiments which were made on the anterior pituitary extract and all were strongly positive; normal males, 9; castrate males, 14; normal females, 11; oöphorectomized females, 14.

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[1] Burn, J. H., and Ling, H. W., *Quart. J. Pharm. and Pharmacol.*, 1933, **6**, 31.

[2] Deuel, H. J., Jr., Grunewald, C. F., and Cutler, C., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 309.

When the anterior pituitary extract was injected in normal male and female rats which were fasted but to which 10% NaCl was administered instead of diacetic acid, the excretion of acetone bodies in the urine was promptly increased from an average of about 1 mg. per day to 30-65 mg. No sexual difference in response was noted. Boiled extract was inactive as was the filtrate after an alcoholic precipitation of the active extract. Negative results were likewise obtained when an active extract was fed by stomach tube instead of being injected subcutaneously.

It seems that the greater susceptibility of the female to ketosis may be associated with a larger production of the ketogenetic substance in the anterior lobe as the result of the stimulation of some substance produced in the ovary other than theelin.

7106 C

Evidence for Characteristic Modifications of the Electrocardiogram Produced by Lesions of Ventricular Muscle Bands.*

JANE SANDS ROBB. (Introduced by M. S. Dooley.)

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Efforts to correlate the electrocardiogram with myocardial lesions as recently reviewed by Haney, Borman, and Meek[1] have not been altogether consistent. In this study individual ventricular muscle bands have been made anemic by ligating their blood supply. The specific muscles were so treated on 26 occasions in 7 dogs, under pento-barbital anesthesia, when the heart was exposed and beating in a snug cradle formed by stitching the pericardium to the edges of the wound. The electrocardiographic results are characteristic and entirely consistent for each of 4 muscles studied; the superficial sino-spiral, the superficial bulbo-spiral, the deep sino-spiral and the deep bulbo-spiral.

When vessel after vessel to a given muscle is ligated, the characteristic result is evident at once, and the magnitude of the effect increases as the lesion of the muscle progresses.

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* This study was made while the author held a grant from the Committee on Scientific Research of the American Medical Association. A part of the experiment was carried out in the Physiological Laboratory of the School of Medicine of Rochester University.

[1] Haney, H. F., Borman, M. C., and Meek, W. J., *Am. J. Physiol.*, 1933, **106**, 64.

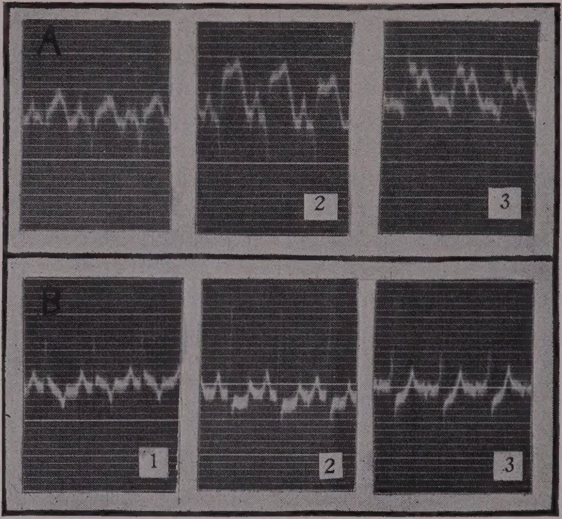

FIG. 1.

(a) Result of lesion in deep bulbo-spiral muscle. Note R-T elevation in all 3 leads. (b) Similar lesion in the deep sino-spiral. Note that in lead I R-T is elevated and T is negative, while in lead III T is positive and S-T is negative.

If 2 adjacent muscles in the same general area of the heart are made anemic, each has its own characteristic effect on the electrocardiogram. Thus, in the base of the left ventricle are 2 muscles, the deep bulbo-spiral and the deep sino-spiral. Defect of the bulbo-spiral will cause an elevation of the R-T segment in all 3 leads. On the other hand, defect of the sino-spiral will cause elevation of the R-T in lead I and depression of the S-T in II and III. Moreover, the same change may be obtained by a lesion in the other ventricle because this same muscle may be damaged either at its origin in the left ventricle or at its insertion in the right ventricle. If both ends of this muscle be injured, the amplitude of the alteration is greater.

The papillary muscles likewise have characteristic effects on the electrocardiogram. Lesion of the anterior papillary muscle of the left ventricle (a part of the superficial bulbo-spiral) produces a sharply negative T in all leads; with depression of S-T in I and an elevation in II and III.

When the posterior papillary muscle (a part of the superficial sino-spiral) is rendered anemic, the T is again negative but the S-T is elevated in all 3 leads.

If more than one muscle is eliminated, of course, the picture is different. For example, the right coronary artery supplies the deep sino-spiral already mentioned and also the superficial sino-spiral and the scroll muscles. Its occlusion causes S-T depression in all 3 leads.

TABLE I.

Muscle	Characteristic Electrocardiogram	
S.B.S.	T very neg. in I, II, III	R-T elevated in I S-T depressed in II, III
D.B.S.	T positive in I, II, III (Fig. Ia)	R-T very elev. in I, II, III
S.S.S.	T slightly neg. in I, II, III	R-T elevated in I, II, III
D.S.S.	T_1 neg. T_3 pos. (Fig. Ib)	R-T elevated in I S-T very depressed in II, III

Table I summarizes the immediate effects of functional elimination of individual muscles.

One may conclude that lesions of the individual muscle bands do cause characteristic electrocardiographic changes.